U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371 AND 37 CFR 1.491

ATTORNEY DOCKET NO.	
401371	
U.S. APPLICATION NO.	
09/937150	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/08231	March 23, 2000	March 23, 1999

TITLE OF INVENTION
PHENYLALANINE DERIVATIVES

APPLICANT(S) FOR DO/EO/US
BURKE, Jr. ET AL.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 USC 371 and 37 CFR 1.491.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371 and 37 CFR 1.491.
3. ☒ This is an express request to begin national examination procedures (35 USC 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 USC 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 USC 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 USC 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 USC 371(c)(5)).
11. Nucleotide and/or Amino Acid Sequence Submission
    a. ☐ Computer Readable Form (CRF)
    b. Specification Sequence Listing on:
       i. ☐ CD-ROM or CD-R (2 copies); or
       ii. ☐ Paper Copy
    c. ☐ Statement verifying identity of above copies

Items 12 to 19 below concern other document(s) or information included:

12. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
    ☐ Form PTO-1449
    ☐ Copies of Listed Documents
13. ☐ An assignment for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ Application Data Sheet Under 37 CFR 1.76
18. ☒ Return Receipt Postcard
19. ☒ Other items or information: Request for Approval of changes to the Drawings

U.S. APPLICATION NO. 09/937150	INTERNATIONAL APPLICATION NO. PCT/US00/08231	ATTORNEY DOCKET NO. 401371

20. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO, but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 710.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1) to (4) ..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT=	$860.00	
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	41 -20=	20	x $ 18.00	$378.00	
Independent Claims	7 - 3 =	4	x $ 80.00	$320.00	
☐ Multiple Dependent Claim(s) (if applicable)			+$270.00	$	

TOTAL OF ABOVE CALCULATIONS=	$1558.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.	$	
SUBTOTAL=	$	
Processing fee of **$130.00** for furnishing English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date.	$	
TOTAL NATIONAL FEE=	$1558.00	
Fee for recording the enclosed assignment. The assignment must be accompanied by an appropriate cover sheet. **$40.00** per property +	$	
TOTAL FEE ENCLOSED=	$1558.00	
	Amount to be: refunded	$
	charged:	$

a. ☒ A check in the amount of $1558.00 to cover the above fee is enclosed.

b. ☐ Please charge Deposit Account No. 12-1216 in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 12-1216. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23548

PATENT TRADEMARK OFFICE

Xavier Pillai

Xavier Pillai, Registration No. 39,799
One of the Attorneys for Applicant(s)

September 21, '01

Date

PATENT
Attorney Docket No. 401371/NIH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

BURKE, Jr., et al.

Application No. Unassigned

Filed: Herewith

Art Unit: Unassigned

Examiner: Unassigned

For: PHENYLALANINE DERIVATIVES

REQUEST FOR APPROVAL OF CHANGES TO THE DRAWINGS

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The Examiner is requested to approve the changes to Figures 2 and 18, as shown in red on the attached sheets of drawings.

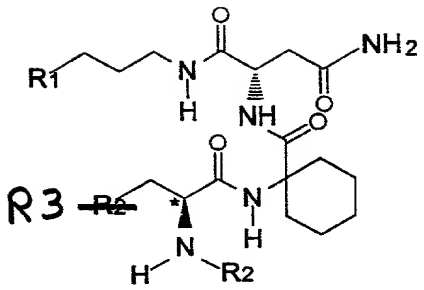
Respectfully submitted,

LEYDIG, VOIT & MAYER, LTD.

Xavier Pillai, Ph.D.
Registration No. 39,799

Suite 300
700 Thirteenth Street, N.W.
Washington, D.C. 20005
Telephone: (202) 737-6770
Facsimile: (202) 737-6776
Date: September 21, '01
XP:jj

Drawings - Amendment (Rev. 8/1/2001)



General Structure
(*D, L and racemate claimed)

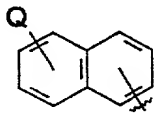
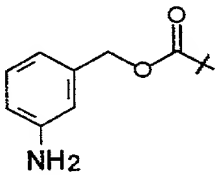
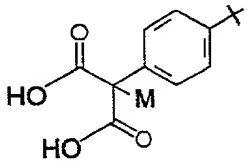
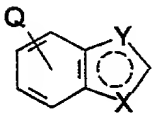
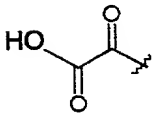
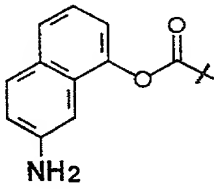
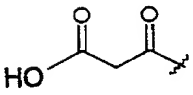
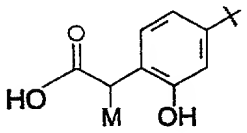
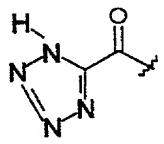
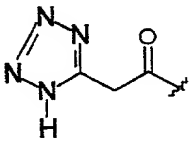
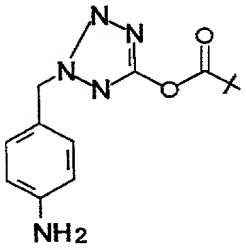
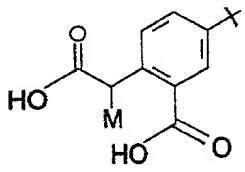
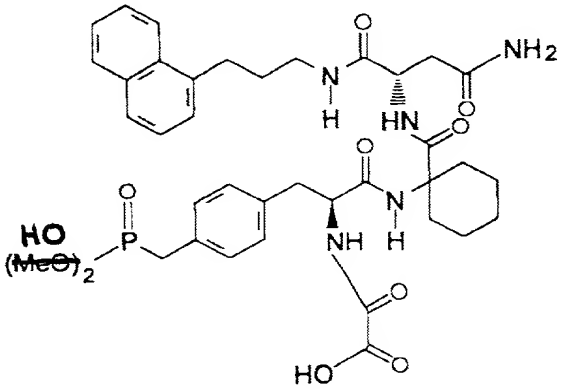
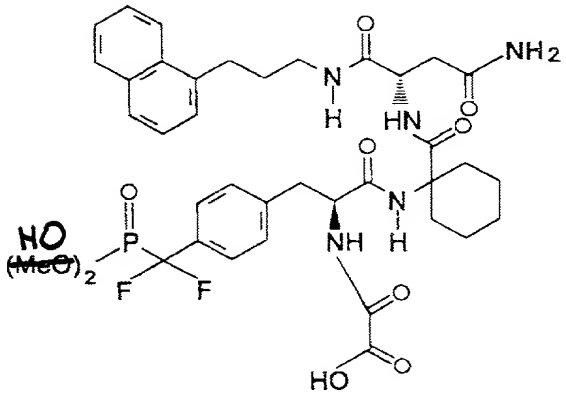
R_1	R_2	R_3
 1 and 2-substitued naphthyl	 	
 where Q=Me, short alkyl, halogen, hydroxy, alkyloxy, amino, acylamino	  	
	  	 where M= hydroxy, alkyloxy, halogen, keto, short alkyl

FIG. 2

FIG. 18



Compd 126



Compd A

PATENT
Attorney Docket No. 401371/NIH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

BURKE, Jr., et al.

Application No. Unassigned

Filed: Herewith

Art Unit: Unassigned

Examiner: Unassigned

For: PHENYLALANINE DERIVATIVES

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination of the patent application, please enter the following amendments and consider the following remarks. Pages showing the requested changes are attached.

IN THE DRAWINGS:

The Examiner is requested to approve the changes to Figures 2 and 18 as indicated in the attached Request for Approval of Drawing Amendments.

IN THE SPECIFICATION:

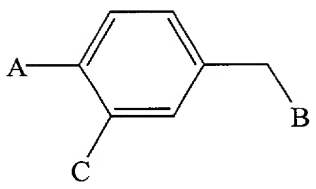
Please insert as the first line at page 1:

This is the national stage of PCT/US00/08231, filed March 23, 2000, which claims the benefit of U.S. Provisional Patent Application No. 60/126,047, filed March 23, 1999, the disclosure of which is incorporated by reference.

IN THE CLAIMS:

Cancel claims 10-23, 25 (both occurrences), 27-28, 31-33, 35-37, 49-65, 68-70, 73-76, 78-83, 86-89, 93-105, 107-111, and 113-114 without prejudice. Replace the indicated claims with:

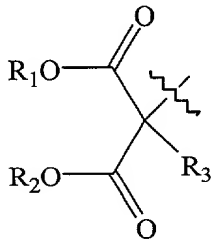
1. (Amended) A compound of formula I:



(I),

wherein:

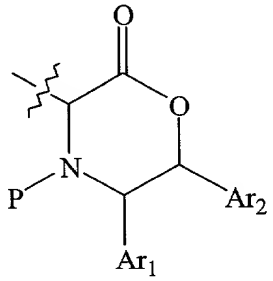
A is carboxyl, carboxyalkyl, dicarboxyalkyl, alkoxycarbonyl, alkoxycarbonylalkyl, dialkoxycarbonylalkyl, or a malonyl group of formula II:



(II),

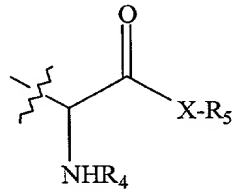
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, alkyl, aryl, and alkoxy;

B has the formula III:



(III),

wherein P is an amine protecting group; and Ar_1 and Ar_2 are aryl groups; or the formula IV:



(IV),

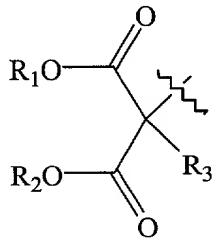
wherein X is NH or O; R_4 is hydrogen, alkyl, aryl, alkylaryl, arylalkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, and alkoxycarbonyl alkyl;

wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of alkyl, hydroxy, halo, keto, amino, and alkoxy; with the provisos that (i) R_5 is not hydrogen when A is carboxyl or carboxyalkyl, C is hydrogen, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is NH; and (ii) R_5 is not hydrogen or alkyl when A is carboxyl or carboxyalkyl, C is hydrogen or hydroxy, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is O.

2. (Amended) The compound of claim 1, wherein:

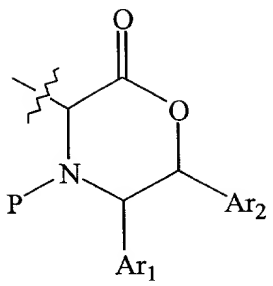
A is carboxyl, carboxyl C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyl, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl, C_1-C_6 dialkoxycarbonyl C_1-C_6 alkyl, or a malonyl group of formula II:



(II),

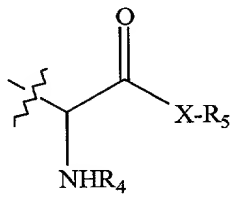
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, C_1-C_6 alkyl, aryl, and C_1-C_6 alkoxy;

B has the formula III:



(III),

wherein P is an amine protecting group; and Ar_1 and Ar_2 are aryl groups; or B has the formula IV:

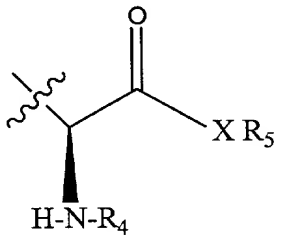


(IV),

wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and

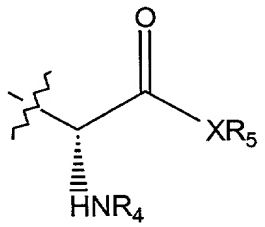
C is selected from the group consisting of hydrogen, hydroxyl, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, C_1-C_6 alkylcarbonyloxy, C_1-C_6 alkoxycarbonyl, and C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl; wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of C_1-C_6 alkyl, hydroxy, halo, keto, amino, and C_1-C_6 alkoxy.

4. (Amended) The compound of claim 3, wherein B has the formula:



wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

5. (Amended) The compound of claim 3, wherein B has the formula:

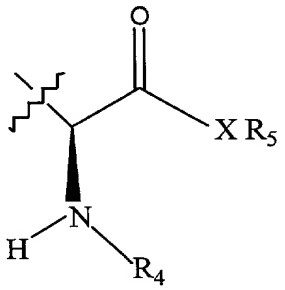


wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

6. (Amended) The compound of claim 4, wherein X is O.

9. (Amended) The compound of claim 8, wherein the amine protecting group is selected from the group consisting of fluorenylmethoxycarbonyl, tert-butoxycarbonyl, carbobenzoxy, and carbamoyl.

24. (Amended) The compound of claim 1, wherein R_1 and R_2 are tert-butyl, R_3 is hydrogen, and B has the formula



wherein X is O, R_4 is fluorenylmethoxycarbonyl, and R_5 is hydrogen.

34. (Amended) A conjugate comprising a conjugant covalently linked to a compound of claim 1.

44. (Amended) The compound of claim 41, wherein E is hydrogen.

46. (Amended) The compound of claim 41, wherein R_3, R_4, R_5, and R_6 are hydrogen.

48. (Amended) The compound of claim 38, wherein W is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S.

66. (Amended) The compound of claim 38, wherein Z is aryl C_1-C_6 alkylamino.

71. (Amended) The compound of claim 38, wherein Z is aryl heterocyclyl C_1-C_6 alkylamino.

77. (Amended) The compound of claim 38, wherein said amino acid is selected from the group consisting of glycine, alanine, valine, norvaline, leucine, iso-leucine, norleucine, α-amino n-decanoic acid, serine, homoserine, threonine, methionine, cysteine, S-acetylaminomethyl-cysteine, proline, trans-3- and trans-4-hydroxyproline, phenylalanine, tyrosine, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, tryptophan, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aspartic acid, asparagine, aminomalonic acid, aminomalonic acid monoamide, glutamic acid, glutamine, histidine, arginine, lysine, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-

diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

84. (Amended) A composition comprising a pharmacologically acceptable carrier and a compound of claim 38.

85. (Amended) A method for inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting an SH2 domain with a compound of claim 38.

90. (Amended) A method for inhibiting SH2 domain binding comprising exposing a material containing an SH2 domain to a compound of claim 38.

91. (Amended) A method for determining the presence of an SH2 domain in a material comprising:
(a) exposing a sample of said material to a SH2 binding compound and obtaining a first binding result;
(b) exposing another sample of said material to a compound of claim 38 and obtaining a second binding result; and
(c) comparing the first and second binding results to determine whether an SH2 domain is present in the material.

92. (Amended) A method of preventing or treating a disease, state, or condition in a mammal comprising administering a compound of claim 38.

106. (Amended) A method of enhancing the therapeutic effect of a treatment rendered to a mammal that has been afflicted with a disease, state, or condition, comprising administering to the mammal a compound of claim 38 in conjunction with the treatment.

112. (Amended) A method of inhibiting the MAP kinase activity in a mammal comprising administering to the mammal a compound of claim 38.

Add the following claims:

115. (New) A compound of the formula:

$$W\text{-}Y\text{-}(AA)_n\text{-}Z$$

wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having (i) dicarboxy C_1-C_6 alkyl , (ii) hydroxyl and carboxy C_1-C_6 alkyl, (iii) carboxyl and carboxy C_1-C_6 alkyl, or (iv) dicarboxyhalo C_1-C_6 alkyl, or dicarboxyhalo C_1-C_6 alkyloxy; or an ester of (i), (ii), (iii), or (iv); wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

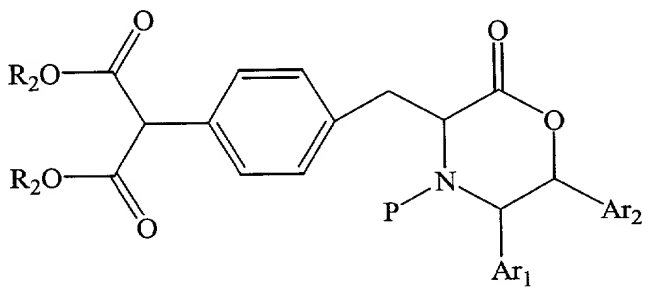
Z is an aryl C_1-C_6 alkylamino or arylheterocyclyl C_1-C_6 alkylamino;

or a salt thereof.

116. (New) A composition comprising a pharmacologically acceptable carrier and a compound of claim 115.

117. (New) A method for inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting an SH2 domain with a compound of claim 115.

118. (New) A process for the preparation of a compound of formula VII:



(VII),

wherein R_2 is alkyl, P is an amine protecting group, and Ar_1 and Ar_2 are aryl; the process comprising:

(a) converting a p-halotoluene to a p-tolyl-malonic acid dialkyl ester by contacting the p-halotoluene with a dialkylmalonate and a cuprous halide;

(b) halogenating the p-tolyl-malonic acid dialkyl ester to obtain a (4-halomethylphenyl)-malonic acid dialkyl ester; and

(c) contacting the (4-halomethylphenyl)-malonic acid ester with a benzyl-6-oxo-2,3-diaryl-4-morpholine to obtain the compound of formula VII.

REMARKS

The specification has been amended to indicate that this application is the national stage of PCT/US00/08231 and to include a reference to the prior provisional application. Claims 6, 24, 34, 44, 46, 48, 66, 71, 77, 84, 85, 90-92, 106, and 112 have been amended to remove multiple dependencies. Claims 1, 2, 4, 5, and 9 have been amended to remove obvious typographical errors. Claim 77 has been amended to remove an obvious editing error. New claims 115-118 have been added and are directed to embodiments of the invention. New claim 118 corresponds to original claim 25 (second occurrence). Claims 1-9, 24, 26, 29-30, 34, 38-48, 66-67, 71-72, 77, 84-85, 90-92, 106, 112, and 115-118 are currently pending. A set of pending claims is attached. Figures 2 and 18 have been amended to correct obvious typographical errors. The drawing amendment is supported, e.g., by the priority application Figures 2 and 18. No new matter has been added by way of this Preliminary Amendment.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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Date: Sept. 21, '01
XP:jj

PATENT
Attorney Docket No. 401371/NIH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

BURKE, Jr., et al.

Application No. Unassigned

Filed: Herewith

For: PHENYLALANINE DERIVATIVES

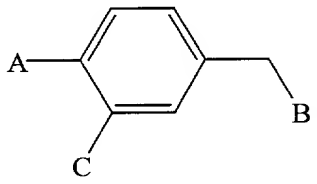
Art Unit: Unassigned

Examiner: Unassigned

AMENDMENTS TO CLAIMS MADE VIA PRELIMINARY AMENDMENT

Amendments to existing claims:

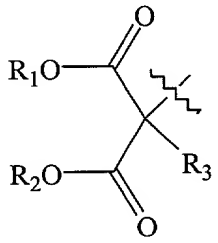
1. (Amended) A compound of formula I:



(I),

wherein:

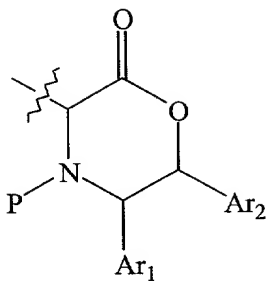
A is carboxyl, carboxyalkyl, dicarboxyalkyl, alkoxycarbonyl, alkoxycarbonylalkyl, dialkoxycarbonylalkyl, or a malonyl group of formula II:



(II),

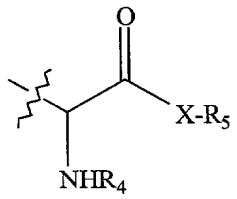
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, alkyl, aryl, and alkoxy;

B has the formula III:



(III),

wherein P is an amine ~~protective~~ protecting group; and Ar_1 and Ar_2 are aryl groups; or the formula IV:



(IV),

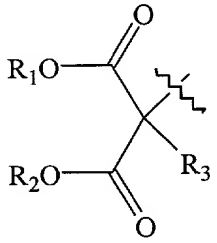
wherein X is NH or O; R_4 is hydrogen, alkyl, aryl, alkylaryl, arylalkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, and alkoxycarbonyl alkyl;

wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of alkyl, hydroxy, halo, keto, amino, and alkoxy; with the provisos that (i) R_5 is not hydrogen when A is carboxyl or carboxyalkyl, C is hydrogen, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is NH; and (ii) R_5 is not hydrogen or alkyl when A is carboxyl or carboxyalkyl, C is hydrogen or hydroxy, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is O.

2. (Amended) The compound of claim 1, wherein:

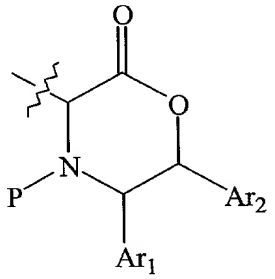
A is carboxyl, carboxyl C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyl, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl, C_1-C_6 dialkoxycarbonyl C_1-C_6 alkyl, or a malonyl group of formula II:



(II),

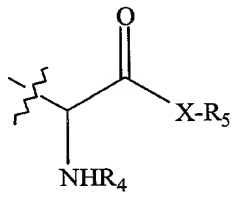
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, C_1-C_6 alkyl, aryl, and C_1-C_6 alkoxy;

B has the formula III:



(III),

wherein P is an amine ~~protective~~ protecting group; and Ar_1 and Ar_2 are aryl groups; or B has the formula IV:

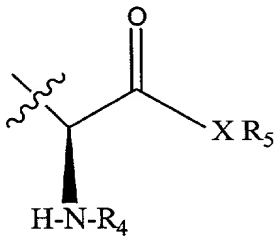


(IV),

wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine ~~protective~~ protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and

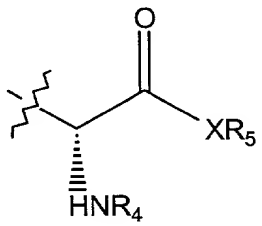
C is selected from the group consisting of hydrogen, hydroxyl, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, C_1-C_6 alkylcarbonyloxy, C_1-C_6 alkoxycarbonyl, and C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl; wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of C_1-C_6 alkyl, hydroxy, halo, keto, amino, and C_1-C_6 alkoxy.

4. (Amended) The compound of claim 3, wherein B has the formula:



wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine ~~protective~~ protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

5. (Amended) The compound of claim 3, wherein B has the formula:

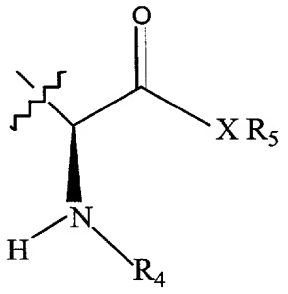


wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine ~~protective~~ protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

6. (Amended) The compound of claim ~~4 or 5~~, wherein X is O.

9. (Amended) The compound of claim 8, wherein ~~acid~~ the amine protecting group is selected from the group consisting of fluorenylmethoxycarbonyl, tert-butoxycarbonyl, carbobenzoxy, and carbamoyl.

24. (Amended) The compound of claim 1 ~~or 2~~, wherein R_1 and R_2 are tert-butyl ~~and~~, R_3 is hydrogen, and B has the formula



wherein X is O, R_4 is fluorenylmethoxycarbonyl, and R_5 is hydrogen.

34. (Amended) A conjugate comprising a conjugant covalently linked to a compound of ~~any of claims 1-25~~ claim 1.

44. (Amended) The compound of ~~any of claims 41-43~~ claim 41, wherein E is hydrogen.

46. (Amended) The compound of ~~any of claim 41-45~~ claim 41, wherein R_3, R_4, R_5, and R_6 are hydrogen.

48. (Amended) The compound of ~~any of claims 38-47~~ claim 38, wherein W is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-

C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S.

66. (Amended) The compound of ~~any of claims 38-65~~ claim 38, wherein Z is aryl C_1-C_6 alkylamino.

71. (Amended) The compound of ~~any of claims 38-65~~ claim 38, wherein Z is aryl heterocyclyl C_1-C_6 alkylamino.

77. (Amended) The compound of ~~any of claims 38-76~~ claim 38, wherein said amino acid is selected from the group consisting of glycine, alanine, valine, norvaline, leucine, iso-leucine, norleucine, α-amino n-decanoic acid, serine, homoserine, threonine, methionine, cysteine, S-acetylaminomethyl-cysteine, proline, trans-3- and trans-4-hydroxyproline, phenylalanine, tyrosine, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, tryptophan, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aspartic acid, asparagine, aminomalonic acid, aminomalonic acid monoamide, glutamic acid, glutamine, histidine, arginine, lysine, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid ~~and~~, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

84. (Amended) A composition comprising a pharmacologically acceptable carrier and a compound of ~~any of claims 38-83~~ claim 38.

85. (Amended) A method for inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting an SH2 domain with a compound of ~~any of claims 34-83~~ claim 38.

90. (Amended) A method for inhibiting SH2 domain binding comprising exposing a material containing an SH2 domain to a compound of ~~any of claims 34-83~~ claim 38.

91. (Amended) A method for determining the presence of an SH2 domain in a material comprising:

(a) exposing a sample of said material to a SH2 binding compound and obtaining a first binding result;
(b) exposing another sample of said material to a compound of ~~any of claims 34-83~~ claim 38 and obtaining a second binding result; and
(c) comparing the first and second binding results to determine whether an SH2 domain is present in the material.

92. (Amended) A method of preventing or treating a disease, state, or condition in a mammal comprising administering a compound of ~~any of claims 34-83~~ claim 38.

106. (Amended) A method of enhancing the therapeutic effect of a treatment rendered to a mammal that has been afflicted with a disease, state, or condition, comprising administering to the mammal a compound of ~~any of claims 38-83~~ claim 38 in conjunction with the treatment.

112. (Amended) A method of inhibiting the MAP kinase activity in a mammal comprising administering to the mammal a compound of ~~any of claims 34-83~~ claim 38.

PATENT
Attorney Docket No. 401371/NIH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

BURKE, Jr., et al.

Application No. Unassigned

Filed: Herewith

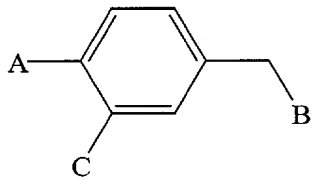
For: PHENYLALANINE DERIVATIVES

Art Unit: Unassigned

Examiner: Unassigned

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

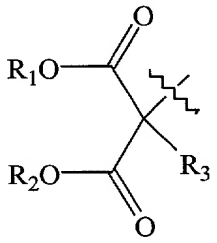
1. A compound of formula I:



(I),

wherein:

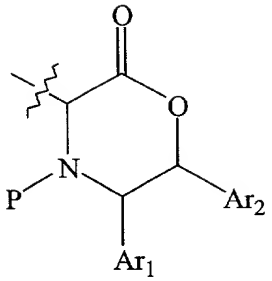
A is carboxyl, carboxyalkyl, dicarboxyalkyl, alkoxycarbonyl, alkoxycarbonylalkyl, dialkoxycarbonylalkyl, or a malonyl group of formula II:



(II),

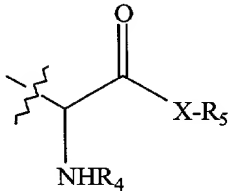
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, alkyl, aryl, and alkoxy;

B has the formula III:



(III),

wherein P is an amine protecting group; and Ar_1 and Ar_2 are aryl groups; or the formula IV:



(IV),

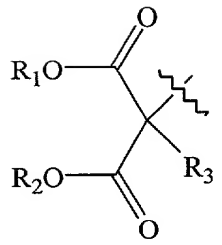
wherein X is NH or O; R_4 is hydrogen, alkyl, aryl, alkylaryl, arylalkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, and alkoxycarbonyl alkyl;

wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of alkyl, hydroxy, halo, keto, amino, and alkoxy; with the provisos that (i) R_5 is not hydrogen when A is carboxyl or carboxyalkyl, C is hydrogen, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is NH; and (ii) R_5 is not hydrogen or alkyl when A is carboxyl or carboxyalkyl, C is hydrogen or hydroxy, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is O.

2. The compound of claim 1, wherein:

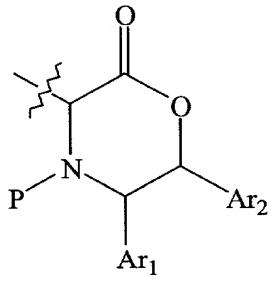
A is carboxyl, carboxyl C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyl, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl, C_1-C_6 dialkoxycarbonyl C_1-C_6 alkyl, or a malonyl group of formula II:



(II),

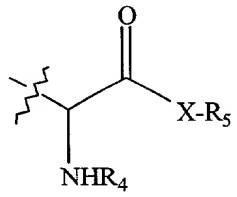
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, C_1-C_6 alkyl, aryl, and C_1-C_6 alkoxy;

B has the formula III:



(III),

wherein P is an amine protecting group; and Ar_1 and Ar_2 are aryl groups; or B has the formula IV:



(IV),

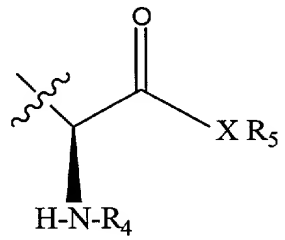
wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, C_1-C_6 alkylcarbonyloxy, C_1-C_6 alkoxycarbonyl, and C_1-C_6 alkoxycarbonyl C_1-

C_6 alkyl; wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of C_1-C_6 alkyl, hydroxy, halo, keto, amino, and C_1-C_6 alkoxy.

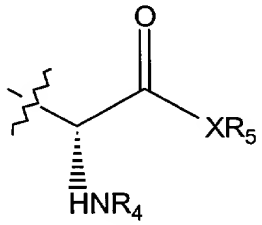
3. The compound of claim 2, wherein B has the formula IV.

4. The compound of claim 3, wherein B has the formula:



wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

5. The compound of claim 3, wherein B has the formula:



wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

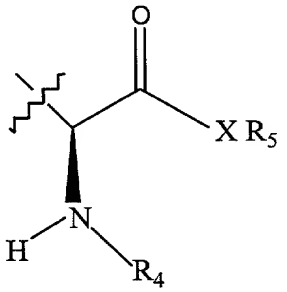
6. The compound of claim 4, wherein X is O.

7. The compound of claim 6, wherein R_4 is hydrogen.

8. The compound of claim 6, wherein R_4 is an amine protecting group.

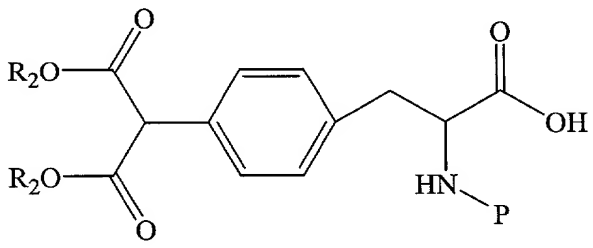
9. The compound of claim 8, wherein the amine protecting group is selected from the group consisting of fluorenylmethoxycarbonyl, tert-butoxycarbonyl, carbobenzoxy, and carbamoyl.

24. The compound of claim 1, wherein R_1 and R_2 are tert-butyl, R_3 is hydrogen, and B has the formula



wherein X is O, R_4 is fluorenylmethoxycarbonyl, and R_5 is hydrogen.

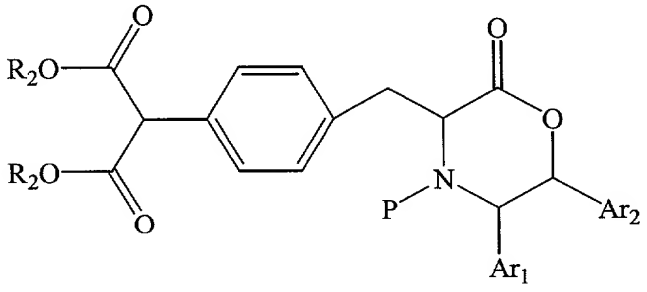
26. A process for preparing a compound of formula VIII:



(VIII),

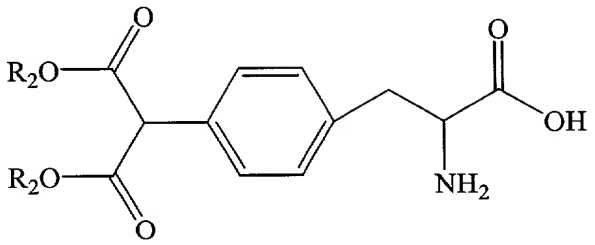
wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing the compound of formula



(VII),

to obtain a compound of formula IX:

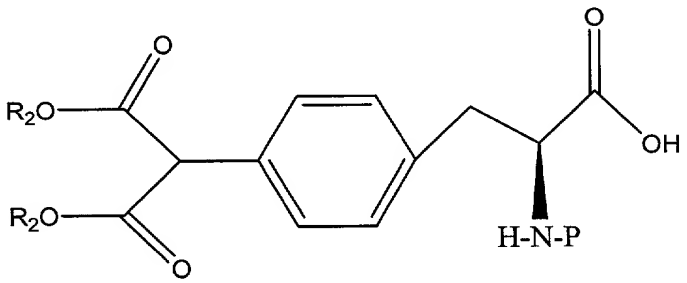


(IX);

and

(b) reacting the compound of formula IX with an amine protecting agent to obtain the compound of formula VIII.

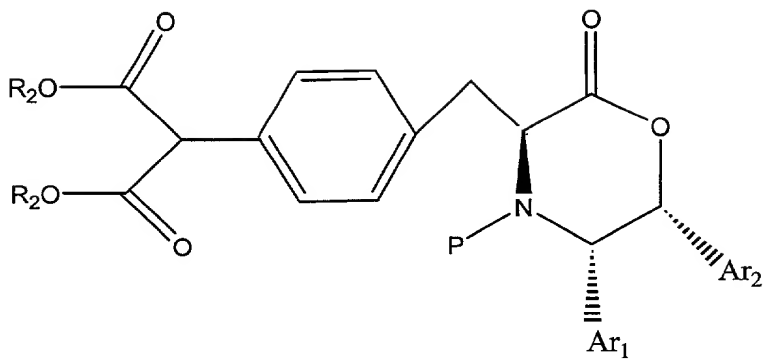
29. A process for preparing a compound of formula VIIIa:



(VIIIa)

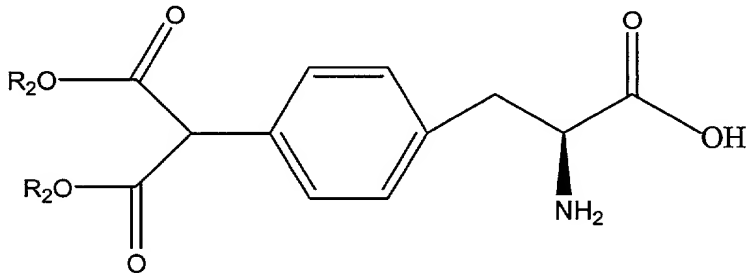
wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing a compound of formula VII



(VIIa)

to obtain a compound of formula IXa:

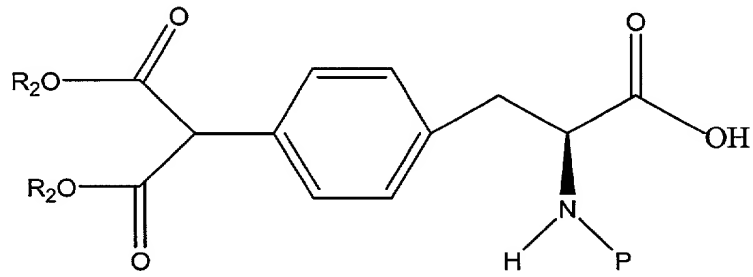


(IXa);

and

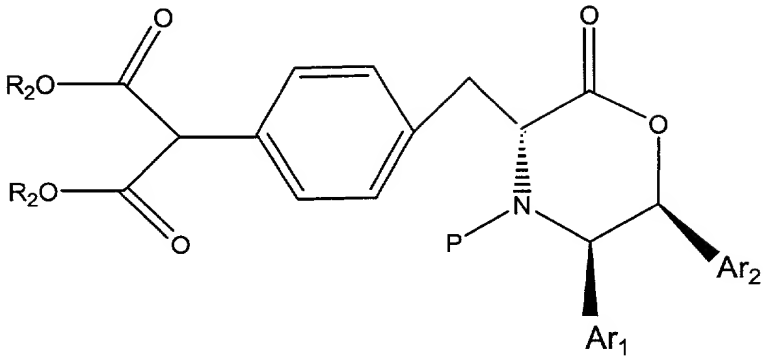
(b) reacting the compound of formula IXa with an amine protecting agent to obtain the compound of formula VIII.

30. A process for preparing a compound of the formula:

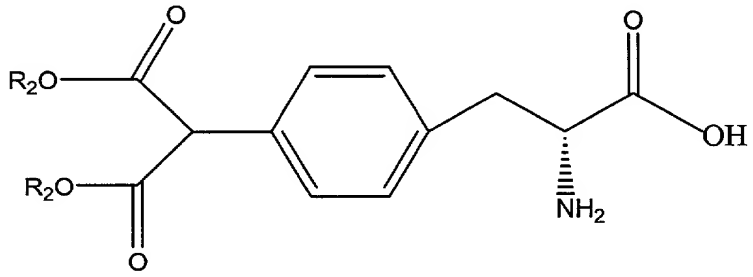


wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing a compound of formula:



to obtain a compound of formula IXb:



(IXb);

and (b) reacting the compound of formula IXa with an amine protecting agent to obtain the compound of formula VIII.

34. A conjugate comprising a conjugant covalently linked to a compound of claim 1.

38. A compound of the formula:

$$W\text{-}Y\text{-}(AA)_n\text{-}Z$$

wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxyalkyl, carboxyalkyloxy, dicarboxyalkyl, dicarboxyalkyloxy, dicarboxyhaloalkyl, dicarboxyhaloalkyloxy, and phosphonoalkyl, phosphonohaloalkyl, wherein the alkyl portion of the substituents may be unsubstituted or

substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of alkylcarbonyl, oxalyl, alkylaminooxalyl, arylaminooxalyl, arylalkylaminooxalyl, alkoxyoxalyl, carboxyalkyl carbonyl, heterocyclyl carbonyl, heterocyclylalkyl carbonyl, arylalkyl heterocyclylalkyl carbonyl, aryloxycarbonyl, and arylalkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an arylalkylamino or arylheterocyclyl alkylamino;

or a salt thereof;

with the proviso that W is not arylalkylamino when the phenyl ring of phenylalanyl contains a phosphonoalkyl or phosphonohaloalkyl substituent at a position para to the alkylamido group and the ortho and meta positions are unsubstituted.

39. The compound of claim 38, wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxy C_1-C_6 alkyl, carboxy C_1-C_6 alkyloxy, dicarboxy C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyloxy, dicarboxyhalo C_1-C_6 alkyl, dicarboxyhalo C_1-C_6 alkyloxy, and phosphono C_1-C_6 alkyl, phosphonohalo C_1-C_6 alkyl, wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy,

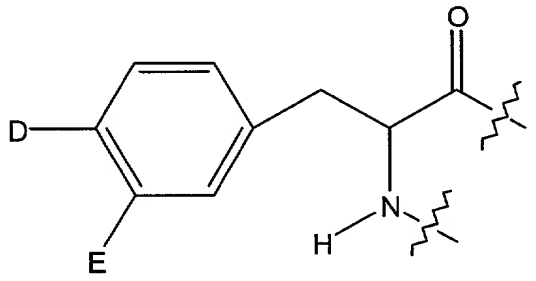
and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an aryl C_1-C_6 alkylamino or arylheterocyclyl C_1-C_6 alkylamino;

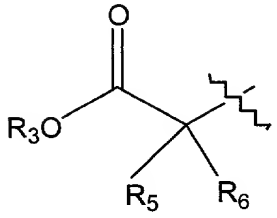
or a salt thereof.

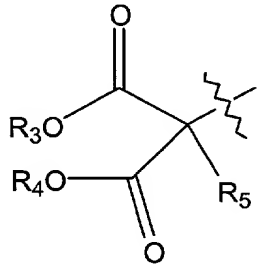
40. The compound of claim 39, wherein Y is of the formula XI:

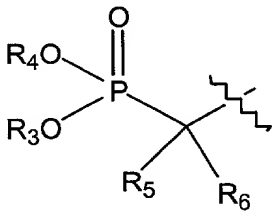


(XI)

wherein D has the formula XII, XIII, or XIV:







(XII) (XIII) (XIV)

wherein R_3 and R_4 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkaryl, and heteroaryl; and R_5 and R_6 may be the same or different and are selected from the group consisting of hydrogen, halo, hydroxy, amino, and C_1-C_6 alkoxy; and

E is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, carboxyl, and C_1-C_6 alkylcarbonyl C_1-C_6 alkyl.

41. The compound of claim 40, wherein D is of formula XII.

42. The compound of claim 40, wherein D is of formula XIII.

43. The compound of claim 40, wherein D is of formula XIV.

44. The compound of claim 41, wherein E is hydrogen.

45. The compound of claim 41, wherein E is carboxyl.

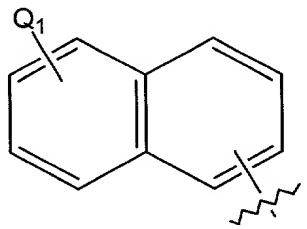
46. The compound of claim 41, wherein R_3, R_4, R_5, and R_6 are hydrogen.

47. The compound of claim 43, wherein R_3 and R_4 are hydrogen.

48. The compound of claim 38, wherein W is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S.

66. The compound of claim 38, wherein Z is aryl C_1-C_6 alkylamino.

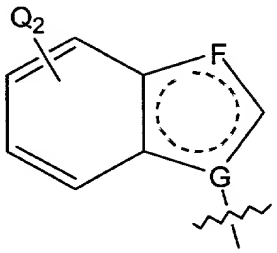
67. The compound of claim 66, wherein the aryl portion of Z has the formula:



wherein Q_1 is hydrogen or a substituent selected from the group consisting of hydroxyl, halo, C_1-C_6 alkyl, C_1-C_6 alkoxy, amino, and C_1-C_6 acylamino.

71. The compound of claim 38, wherein Z is aryl heterocyclyl C_1-C_6 alkylamino.

72. The compound of claim 71, wherein the heterocyclyl portion of Z has the formula:



wherein Q_2 is hydrogen or a substituent selected from the group consisting of hydroxyl, halo, C_1-C_6 alkyl, C_1-C_6 alkoxy, amino, and C_1-C_6 acylamino, and F and G are independently selected from the group consisting of C, N, O, and S.

77. The compound of claim 38, wherein said amino acid is selected from the group consisting of glycine, alanine, valine, norvaline, leucine, iso-leucine, norleucine, α-amino n-decanoic acid, serine, homoserine, threonine, methionine, cysteine, S-acetylaminomethyl-cysteine, proline, trans-3- and trans-4-hydroxyproline, phenylalanine, tyrosine, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, tryptophan, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aspartic acid, asparagine, aminomalonic acid, aminomalonic acid monoamide, glutamic acid, glutamine, histidine, arginine, lysine, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

84. A composition comprising a pharmacologically acceptable carrier and a compound of claim 38.

85. A method for inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting an SH2 domain with a compound of claim 38.

90. A method for inhibiting SH2 domain binding comprising exposing a material containing an SH2 domain to a compound of claim 38.

91. A method for determining the presence of an SH2 domain in a material comprising:
(a) exposing a sample of said material to a SH2 binding compound and obtaining a first binding result;
(b) exposing another sample of said material to a compound of claim 38 and obtaining a second binding result; and
(c) comparing the first and second binding results to determine whether an SH2 domain is present in the material.

92. A method of preventing or treating a disease, state, or condition in a mammal comprising administering a compound of claim 38.

106. A method of enhancing the therapeutic effect of a treatment rendered to a mammal that has been afflicted with a disease, state, or condition, comprising administering to the mammal a compound of claim 38 in conjunction with the treatment.

112. A method of inhibiting the MAP kinase activity in a mammal comprising administering to the mammal a compound of claim 38.

115. A compound of the formula:

$$W\text{-}Y\text{-}(AA)_n\text{-}Z$$

wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having (i) dicarboxy C_1-C_6 alkyl , (ii) hydroxyl and carboxy C_1-C_6 alkyl, (iii) carboxyl and carboxy C_1-C_6 alkyl, or (iv) dicarboxyhalo C_1-C_6 alkyl, or dicarboxyhalo C_1-C_6 alkyloxy; or an ester of (i), (ii), (iii), or (iv); wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy,

and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

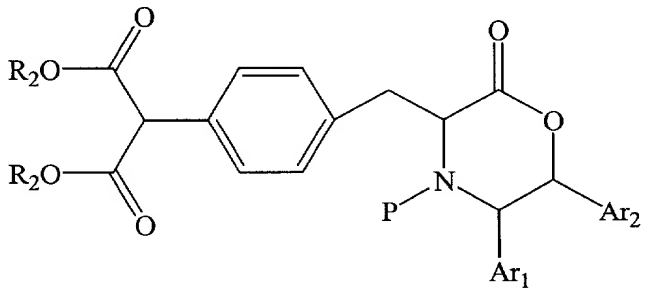
Z is an aryl C_1-C_6 alkylamino or arylheterocyclyl C_1-C_6 alkylamino;

or a salt thereof.

116. A composition comprising a pharmacologically acceptable carrier and a compound of claim 115.

117. A method for inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting an SH2 domain with a compound of claim 115.

118. A process for the preparation of a compound of formula VII:



(VII),

wherein R_2 is alkyl, P is an amine protecting group, and Ar_1 and Ar_2 are aryl; the process comprising:

(a) converting a p-halotoluene to a p-tolyl-malonic acid dialkyl ester by contacting the p-halotoluene with a dialkylmalonate and a cuprous halide;

(b) halogenating the p-tolyl-malonic acid dialkyl ester to obtain a (4-halomethylphenyl)-malonic acid dialkyl ester; and

(c) contacting the (4-halomethylphenyl)-malonic acid ester with a benzyl-6-oxo-2,3-diaryl-4-morpholine to obtain the compound of formula VII.

PHENYLALANINE DERIVATIVES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to novel phenylalanine derivatives, compositions, and a method of using these derivatives in inhibiting SH2 domain binding with a phosphoprotein. The present invention further provides precursors suitable for preparing the phenylalanine derivatives.

BACKGROUND OF THE INVENTION

The pharmaceutical industry is in search for new classes of compounds for the therapy and prophylaxis of proliferative diseases such as cancer, autoimmune diseases, and hyperproliferative skin disorders such as psoriasis. These diseases or disorders affect a large portion of the population, leading to suffering and possibly death.

Some of these diseases or disorders may involve signal transduction. Signal transduction is critical to normal cellular homeostasis and is the process of relaying extracellular messages, e.g., chemical messages in the form of growth factors, hormones and neurotransmitters, via receptors, e.g., cell-surface receptors, to the interior of the cell. Protein-tyrosine kinases play a central role in this biological function. Among others, these enzymes catalyze the phosphorylation of specific tyrosine residues to form tyrosine phosphorylated residues. Examples of this class of enzymes include the PDGF receptor, the FGF receptor, the HGF receptor, members of the EGF receptor family such as the EGF receptor, erb-B2, erb-B3 and erb-B4, the src kinase family, Fak kinase and the Jak kinase family. The tyrosine-phosphorylated proteins are involved in a range of metabolic processes, from proliferation and growth to differentiation.

Protein-tyrosine phosphorylation is known to be involved in modulating the activity of some target enzymes as well as in generating specific complex

networks involved in signal transduction via various proteins containing a specific amino acid sequence called a Src homology region or SH2 domain (see Proc. Natl. Acad. Sci. USA, 90, 5891 (1990)). A malfunction in this protein-tyrosine phosphorylation through tyrosine kinase overexpression or deregulation is manifested by various oncogenic and (hyper-)proliferative disorders such as cancer, inflammation, autoimmune disease, hyperroliferative skin disorders, such as psoriasis, and allergy/asthma.

SH2- and/or SH3- comprising proteins that play a role in cellular signaling and transformation include, but are not limited to, the following: Src, Lck, Eps, ras GTPase-activating protein (GAP), phospholipase C, phosphoinositol-3 (PI-3)kinase, Fyn, Lyk, Fgr, Fes, ZAP-70, Sem-5, p85, SHPTP1, SHPTP2, corkscrew, Syk, Lyn, Yes, Hck, Dsrc, Tec, Atk/Bpk, Itk/Tsk, Arg, Csk, tensin, Vav, Emt, Grb2, BCR-Abl, Shc, Nck, Crk, CrkL, Syp, Blk, 113TF, 91TF, Tyk2, esecially Src, phospholipase c, phoshoinositol-3 (pI-3)kinase, Grb2, BCR-Abl, Shc, Nck, Crk, CrkL, Syp, Blk, 113TF, 91TF, and Tyk2. A direct link has been established between activated receptor kinases and Ras with the finding that the mammalian Grb2 protein, a 26 kilodalton (kD) protein comprising a single SH2 and two SH3 domains bind to proline-rich sequences present in the Sos exchange factor.

The significance of ras-regulatory proteins in human tumors is also highlighted by the critical role of Grb2 in BCR-Abl mediated oncogenesis (J. Exp. Med., 179, 167-175 (1994)).

Central to the binding of SH2 domains with phosphotyrosine ("ptyr") containing ligands is the interaction of the doubly ionized ptyr phosphate with two invariant arginine residues in a well formed pocket. These arginine-phosphate interactions are particularly critical to the overall binding, such that high affinity binding is usually lost by removal of the phosphate group.

Although the ptyr pharmacophore plays a dominant role in SH2 domain-ligand interactions, ptyr residues are not suitable components of inhibitors intended for *in vivo* application, due to the enzymatic lability of the phosphate ester bond and the poor cellular penetration of doubly ionized phosphate species.

In view of the foregoing, there exists a need for molecules that have an ability to mimic the structure of the phosphotyrosine peptide binding site, as

well as a need for compounds that have the ability to disrupt the interaction between SH2 domains of proteins (e.g., regulatory proteins) for example that of Grb2, and proteins with phosphorylated moieties. There also exists a need for suitable starting materials or precursors in the synthesis of the molecules that inhibit binding of SH2 domains. There further exists a need for compounds suitable for use in the therapy or prophylaxis of proliferative diseases or conditions, as well as in diagnosis, assays, and testing.

These advantages of the present invention will be apparent from the detailed description of the embodiments of the invention set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 schematically depicts a route for preparing compounds **1-5**.

Fig. 2 depicts some embodiments of the compounds of formula X in accordance with the present invention.

Fig. 3 depicts some other embodiments of the compounds of formula X in accordance with the present invention, particularly compounds **18-20**.

Figs. 4-13 depict reaction schemes for preparing compounds **8-17**, respectively.

Fig. 14 depicts a reaction scheme for preparing compounds **21-25**.

Fig. 15 depicts a reaction scheme for preparing compounds **26-32**.

Fig. 16 depicts a reaction scheme for preparing compounds **33-38**.

Figs. 17 a-c depict the human breast cancer cell growth and proliferation inhibition by compounds **11**, **12**, **36**, and **38**. Fig. 17a depicts the proliferation inhibition of human breast cancer cells MDA-MB-453; Fig. 17b depicts growth inhibition of MDA-MB-453; and Fig. 17c depicts a lack of growth inhibition of MDA-MB-253 breast cancer cells.

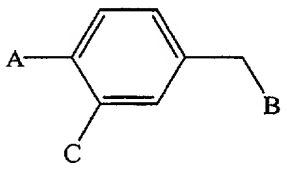
Fig. 18 depicts the formulas of compounds #126 and A referred to in Fig. 17.

Fig. 19 depicts the results of an ELISA assay and shows that the compounds of the present invention have Grb2 inhibitory effect.

Fig. 20 depicts the synergistic effect of a combination treatment of a Grb2 inhibitor (compound #126) with certain chemotherapy drugs.

BRIEF SUMMARY OF THE INVENTION

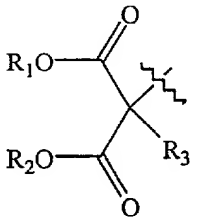
The present invention provides a compound of formula I:



(I),

wherein:

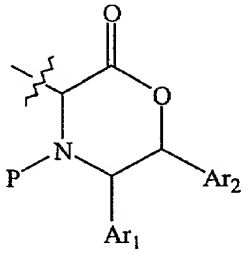
A is carboxyl, carboxyalkyl, dicarboxyalkyl, alkoxycarbonyl, alkoxycarbonylalkyl, dialkoxycarbonylalkyl, or a malonyl group of formula II:



(II),

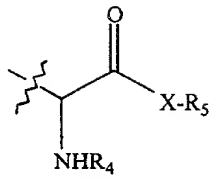
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, alkyl, aryl, and alkoxy;

B has the formula III: B has the formula III:



(III),

wherein P is an amine protecting group; and Ar_1 and Ar_2 are aryl groups; or the formula IV:



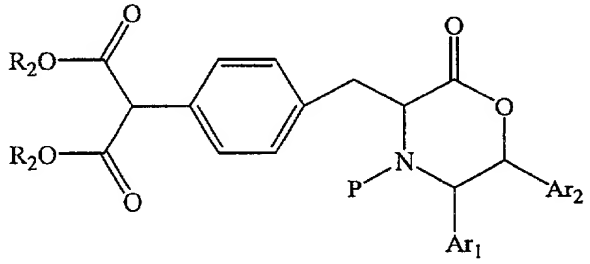
(IV),

wherein X is NH or O; R_4 is hydrogen, alkyl, aryl, alkylaryl, arylalkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, and alkoxycarbonyl alkyl;

wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of alkyl, hydroxy, halo, keto, amino, and alkoxy; with the provisos that (i) R_5 is not hydrogen when A is carboxyl or carboxyalkyl, C is hydrogen, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is NH; and (ii) R_5 is not hydrogen or alkyl when A is carboxyl or carboxyalkyl, C is hydrogen or hydroxy, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is O.

The present invention further provides a process for the preparation of a compound of formula VII:



(VII),

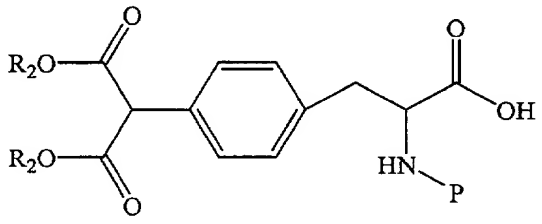
wherein R_2 is alkyl, P is an amine protecting group, Ar_1 and Ar_2 are aryl; the process comprising:

(a) converting a p-halotoluene to a p-tolyl-malonic acid dialkyl ester by contacting the p-halotoluene with a dialkylmalonate and a cuprous halide;

(b) halogenating the p-tolyl-malonic acid dialkyl ester to obtain a (4-halomethylphenyl)-malonic acid dialkyl ester; and

(c) contacting the (4-halomethylphenyl)-malonic acid dialkyl ester with a benzyl-6-oxo-2,3-diaryl-4-morpholine to obtain the compound of formula VII.

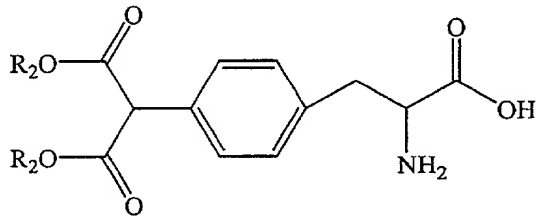
The present invention further provides a process for preparing a compound of formula VIII:



(VIII),

wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing the compound of formula VII to obtain a compound of formula IX



(IX);

and (b) reacting the compound of formula IX with an amine protecting agent to obtain the compound of formula VIII.

The present invention further provides a conjugate comprising a conjugant covalently linked to the compounds of formula I.

The present invention further provides a compound of formula X:

$$W\text{-}Y\text{-}(AA)_n\text{-}Z \qquad (X)$$

wherein n is 0 to 15, Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, and the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxyalkyl, carboxyalkyloxy, dicarboxyalkyl, dicarboxyalkyloxy, dicarboxyhaloalkyl, dicarboxyhaloalkyloxy, and phosphonoalkyl, phosphonohaloalkyl, wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkyloxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of alkylcarbonyl, oxalyl, carboxyalkyl carbonyl, heterocyclyl carbonyl, heterocyclylalkyl carbonyl, arylalkyl heterocyclylalkyl carbonyl, aryloxycarbonyl, and arylalkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an arylalkylamino or arylheterocyclyl alkylamino;

or a salt thereof;

with the proviso that W is not arylalkylamino when the phenyl ring of phenylalanyl contains a phosphonoalkyl or phosphonohaloalkyl substituent at a position para to the alkylamido group and the ortho and meta positions are unsubstituted.

The present invention further provides (a) a composition comprising a pharmacologically acceptable carrier and a compound of formula X, (b) a method of inhibiting an SH2 domain from binding with a protein such as a phosphoprotein comprising contacting an SH2 domain or a sample or substance containing an SH2 domain with a compound of formula X, and (c) compound of formula X for use in medicine. Compounds of formula X find use in the manufacture of a medicament for the treatment of a condition that responds to the inhibition of phosphoprotein binding to an SH2 domain of a

mammal. The present invention further provides a method for determining the presence of an SH2 domain in a material comprising:

(a) exposing a sample of the material to a SH2 binding compound and obtaining a first binding result;

(b) exposing another sample of the material to a conjugate or compound of formula X and obtaining a second binding result; and

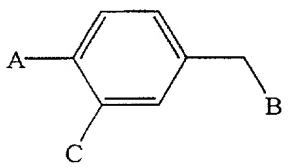
(c) comparing the first and second binding results to determine whether an SH2 domain is present in the material.

The present invention further provides a method of preventing or treating a disease, state, or condition in a mammal that involves an SH2 domain binding comprising administering to the mammal a compound of the present invention. The present invention further provides a method of enhancing the therapeutic effect of a treatment rendered to a mammal that has been afflicted with a disease, state, or condition, comprising administering to the mammal a compound of the present invention in conjunction with the treatment.

While the invention has been described and disclosed below in connection with certain embodiments and procedures, it is not intended to limit the invention to those specific embodiments. Rather it is intended to cover all such alternative embodiments and modifications as fall within the spirit and scope of the invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention provides certain novel phenylalanine conjugates useful in a variety of applications, especially in the treatment or prophylaxis of various diseases or conditions in a mammalian body. Particular examples of such conjugates are phenylalanine peptide conjugates. The present invention further provides phenyl alanine precursors that can be conveniently used in the synthesis of phenylalanine peptide conjugates. Thus, the present invention provides a precursor compound of formula I:



(I),

C is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, and alkoxycarbonyl alkyl;

wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of alkyl, hydroxy, halo, keto, amino, and alkoxy; with the provisos that (i) R_5 is not hydrogen when A is carboxyl or carboxyalkyl, C is hydrogen, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is NH; and (ii) R_5 is not hydrogen or alkyl when A is carboxyl or carboxyalkyl, C is hydrogen or hydroxy, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is O.

The alkyl portion of the various groups described above can have any suitable number of carbon atoms, e.g., from 1 to about 12 carbon atoms, preferably from 1 to 6 carbon atoms, and more preferably from 1 to 4 carbon atoms. The aryl portion of the various groups described can have any number of aromatic rings, e.g., from 1 to 3 rings, preferably 1 or 2 rings, and more preferably 1 ring. Thus, for example, the present invention provides a compound of formula I wherein:

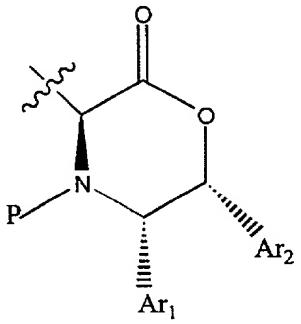
A is carboxyl, carboxyl C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyl, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl, C_1-C_6 dialkoxycarbonyl C_1-C_6 alkyl, or a malonyl group of formula II wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, C_1-C_6 alkyl, aryl, and C_1-C_6 alkoxy;

B has the formula III wherein P is an amine protecting group; and Ar_1 and Ar_2 are aryl groups; or the formula IV, wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and

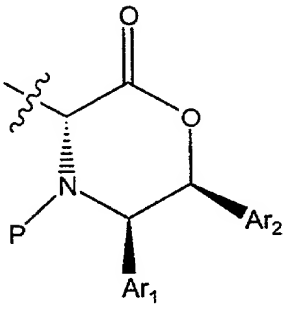
C is selected from the group consisting of hydrogen, hydroxyl, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, C_1-C_6 alkylcarbonyloxy, C_1-C_6 alkoxycarbonyl, and C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl; wherein the aryl, heteroaryl, and the aryl portion of the arylalkyl and alkylaryl may be unsubstituted or substituted with

a substituent selected from the group consisting of C_1-C_6 alkyl, hydroxy, halo, keto, amino, and C_1-C_6 alkoxy.

The asymmetric carbons in B can have any suitable configuration. Specifically, B can have the R, S, or R and S configurations. Thus, B in formula III can have the following structures:



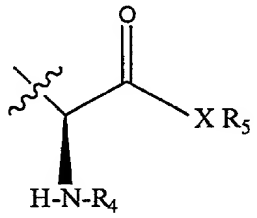
(IIIa)



(IIIb)

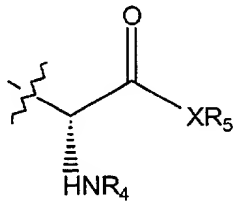
In certain embodiments of the compounds of formula I, wherein B has the formula III, Ar_1 and Ar_2 are phenyl which may be substituted optionally with alkyl, hydroxy, halo, amino, aminoalkyl, or alkoxy substituents. P is an amine protecting group. Any suitable amine protecting group known to those of skill in the art can be used, and for example, the amine protecting group is selected from the group consisting of fluorenylmethoxycarbonyl (Fmoc), tert-butoxycarbonyl (t-Boc), carbobenzoxy (Cbz), and carbamoyl. Preferably, the amine protecting group is Fmoc, t-Boc, or Cbz, and more preferably, Fmoc.

B of formula IV can have the following structures



(IVa),

and



(IVb)

wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protecting group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl. The compound having B of formula IVa is preferred.

In some preferred embodiments, the compound of formula I has the formula IVa and X is O. R_4 is hydrogen in certain embodiments. In some preferred embodiments of the compounds of formula I, B has the formula IVa, X is O, and R_4 is an amine protecting group. Any suitable amine protecting

group known to those of skill in the art can be used, and for example, the amine protecting group is selected from the group consisting of Fmoc, t-Boc, Cbz, and carbamoyl. Preferably, the amine protecting group is Fmoc, t-Boc, or Cbz, and more preferably, Fmoc.

In some embodiments of the compounds of formula I, B has the formula IVa or IVb, X is NH or O, and R_4 is an amine protecting group R_5 is hydrogen. In certain preferred embodiments of the compounds of formula I, B has the formula IVa or IVb, X is NH or O, R_4 is an amine protecting group R_5 is hydrogen, and A is a malonyl group of formula II wherein R_1 and R_2 are hydrogen. It is further preferred that R_3 is hydrogen.

In certain embodiments of the compound of formula I, C is hydrogen. In some embodiments of the compound of formula I, C is C_1-C_6 alkyloxycarbonyl, for example, C is t-Boc. Thus, certain embodiments of the present invention include compounds of formula I wherein B has the formula IVa or IVb, X is NH or O, R_4 is an amine protecting group, R_5 is hydrogen, A is a malonyl group of formula II wherein R_1 and R_2 are hydrogen, and C is hydrogen or C_1-C_6 alkyloxycarbonyl.

In some embodiments of the compound of formula I, C is C_1-C_6 alkylcarbonyloxy, for example, C is acetyloxy. Thus, certain embodiments of the present invention include compounds of formula I wherein B has the formula IVa or IVb, X is NH or O, R_4 is an amine protecting group, R_5 is hydrogen, A is a malonyl group of formula II wherein R_1 and R_2 are hydrogen, and C is C_1-C_6 alkyloxycarbonyl.

Preferred examples of compounds of formula I include a compound wherein A has the formula II wherein R_1 and R_2 are tert-butyl and R_3 is hydrogen, C is hydrogen, and B has the formula IVa wherein X is O, R_4 is Fmoc, and R_5 is hydrogen; a compound wherein A is t-butoxycarbonylmethyl, C is t-butoxycarbonyl, and B has the formula IVa wherein X is O, R_4 is Fmoc, and R_5 is hydrogen; and a compound wherein A is t-butoxycarbonylmethyl, C is acetoxy, and B has the formula IVa wherein X is O, R_4 is Fmoc, and R_5 is hydrogen.

The compounds of formula I can be prepared by processes known to those skilled in the art. The present invention provides a process for preparing compounds of formula I, particularly a compound of formula VIII.

The present invention provides a process for preparing a compound of formula VIII wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing the compound of formula (VII), wherein P is an amine protecting group to obtain a compound of formula IX; and (b) reacting the compound of formula IX with an amine protecting reagent to obtain the compound of formula VIII.

The present invention further provides a process for preparing a compound of formula VII, wherein R_2 is alkyl; the process comprising:

(a) converting a p-halotoluene to a p-tolyl-malonic acid dialkyl ester by contacting the p-halotoluene with a dialkylmalonate and a cuprous halide;

(b) halogenating the p-tolyl-malonic acid dialkyl ester to obtain a (4-halomethylphenyl)-malonic acid dialkyl ester; and

(c) contacting the (4-halomethylphenyl)-malonic acid dialkyl ester with a benzyl-6-oxo-2,3-diaryl-4-morpholine to obtain the compound of formula VII.

An embodiment of the process of the present invention is schematically illustrated in Fig. 1. Thus, for example, p-iodotoluene is contacted with di-tert-butyl malonate and cuprous chloride in the presence of a base such as sodium hydride. The reaction, which produces a p-tolyl-malonic acid dialkyl ester, is carried out in a solvent, preferably a dry polar solvent, such as a solvent including dioxane and hexamethyl phosphoramide (HMPA), at a temperature of from about 80 to about 120°C, and preferably from about 90 to about 110°C. At the end, the reaction mixture is cooled to room temperature (20-25°C), followed by quenching with an ammonium salt. The dialkyl ester is then isolated from the reaction mixture. Preferably, the reaction mixture is extracted with an extracting solvent such as ethyl acetate, washed with brine, and dried. The extracting solvent is then removed, e.g., by distillation, and the resulting dialkyl ester is purified on a chromatographic column.

The p-tolyl-malonic acid dialkyl ester prepared as above can be halogenated by known halogenating agents, e.g., N-bromosuccinimide (NBS). Thus, the p-tolyl-malonic acid dialkyl ester is combined with NBS and a peroxide in a suitable solvent such as CCl_4. The reaction is carried out at an

elevated temperature, preferably at the reflux temperature of the solvent. The precipitates that form are removed, and the desired product is isolated from the liquid portion of the reaction mixture. The liquid portion can be extracted with a non-polar solvent, preferably repeatedly, and the combined non-polar solvent extract is dried. The resulting product, (4-bromophenyl) malonic acid dialkyl ester, is preferably purified on a chromatographic column.

The (4-bromophenyl) malonic acid dialkyl ester prepared as above can be contacted with a benzyl-6-oxo-5,6-diaryl-4-morpholine-carboxylate in the presence of a suitable base to produce a benzyl-3-[dialkyloxycarbonylmethyl)phenylmethyl]-6-oxo-5,6-diaryl-4-morpholine-carboxylate. A polar solvent such as tetrahydrofuran containing HMPA is used for carrying out the reaction. The reaction is carried out at a low temperature, preferably at about -78°C. Lithium bis(trimethylsilyl)amide is an example of a suitable base. After the reaction is complete, the reaction mixture is quenched with an ammonium salt solution. The desired product is extracted, preferably repeatedly, into a non-polar solvent such as ethyl acetate. The non-polar solvent extract is washed with water, dried, and concentrated. The product can be purified on a chromatographic column.

4-(dialkyloxycarbonylmethyl)-phenylalanine can be prepared by reducing the benzyl-3-[dialkyloxycarbonylmethyl)phenylmethyl]-6-oxo-5,6-diaryl-4-morpholine-carboxylate. The reduction can be carried out by hydrogen and a suitable catalyst, e.g., by using hydrogen and palladium black. The reduction can be carried out in a solvent such as tetrahydrofuran-ethanol mixture containing a small amount of acid such as acetic acid. The hydrogen pressure can be maintained at about 45 to about 25 psi, and the reduction can be carried out at room temperature. The resulting product can be purified by washing with an ether. The product of desired stereochemistry can be obtained by the choice of the stereochemistry of the benzyl -6-oxo-2,3-diphenyl-4-morpholine. Thus, benzyl-3-[dialkyloxycarbonylmethyl)phenylmethyl]-6-oxo-5,6-diaryl-4-morpholine-carboxylate can be obtained by choosing benzyl (2R,3S) (-) -6-oxo-2,3-diphenyl-4-morpholine.

N-Fmoc-4-(dialkyloxycarbonylmethyl)-phenylalanine can be prepared by reacting 4-(dialkyloxycarbonylmethyl)-phenylalanine with an amine

protecting agent, e.g., Fmoc-OSu. A base such as sodium bicarbonate can be used to carry out the reaction. The reaction can be carried out in a solvent, e.g., a solvent containing dioxane and water, at room temperature. The reaction is carried out until completion. At the end of the reaction, the reaction mixture is cooled, preferably to 0°C, and acidified. The product is then extracted into an organic solvent, e.g., ethyl acetate, preferably repeatedly. The combined organic extract is washed with water, dried, and concentrated. The resulting crude product can be purified on a chromatographic column, e.g., a silica gel column.

The present invention further provides a conjugate comprising a conjugant covalently linked to a compound of formula I. The conjugate of the present invention can find particular use in the treatment or prophylaxis of various diseases or conditions in a mammal, preferably a human, wherein the phenylalanyl moiety of the conjugate interacts or facilitates interaction, or blocks other ligands from interacting or binding, with a domain or receptor site responsible for the onset or development of a disease or condition. Examples of such domains include the SH2 domains. Examples of such diseases or conditions include proliferative diseases such as cancer and autoimmune diseases. The conjuates also find use in diagnosis, assay, screening, and testing.

The conjugant can be any suitable material that provides a conjugate as described above. The conjugant can be an amino acid or nucleic acid, for example, a natural or synthetic peptide or nucleotide. The conjugant can be a natural or synthetic polymer, for example, carbohydrate polymers. A preferred conjugate is one that contains an amino acid, for example, the conjuates of formula X below.

Accordingly, the present invention provides a compound of the formula:

$$W\text{-}Y\text{-}(AA)_n\text{-}Z \qquad (X)$$

wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxyalkyl, carboxyalkyloxy, dicarboxyalkyl, dicarboxyalkyloxy, dicarboxyhaloalkyl, dicarboxyhaloalkyloxy, and phosphonoalkyl, phosphonohaloalkyl, wherein the

alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of alkylcarbonyl, oxalyl, alkylaminooxalyl, arylaminooxalyl, aryl alkylaminooxalyl, alkoxyoxalyl, carboxyalkyl carbonyl, heterocyclyl carbonyl, heterocyclylalkyl carbonyl, arylalkyl heterocyclylalkyl carbonyl, aryloxycarbonyl, and arylalkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an arylalkylamino or arylheterocyclyl alkylamino;

or a salt thereof;

with the proviso that W is not arylalkylamino when the phenyl ring of phenylalanyl contains a phosphonoalkyl or phosphonohaloalkyl substituent at a position para to the alkylamido group and the ortho and meta positions are unsubstituted.

The alkyl portion of the various groups described above can have any suitable number of carbon atoms, e.g., from 1 to about 12 carbon atoms, preferably from 1 to 6 carbon atoms, and more preferably from 1 to 4 carbon atoms. The aryl portion of the various groups described can have any number of aromatic rings, e.g., from 1 to 3 six rings, preferably 1 or 2 rings, and more preferably 1 ring. Thus, for example, the present invention provides a compound of formula XI wherein Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxy C_1-C_6 alkyl, carboxy C_1-C_6 alkyloxy, dicarboxy C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyloxy, dicarboxyhalo C_1-C_6 alkyl, dicarboxyhalo C_1-C_6 alkyloxy, and phosphono C_1-C_6 alkyl, phosphonohalo C_1-C_6 alkyl, wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent

selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto;

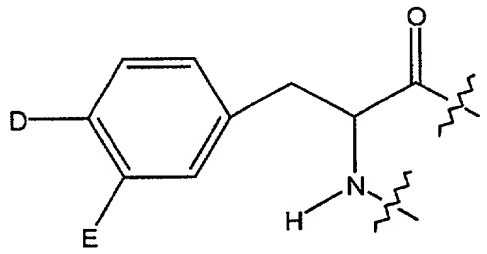
W is a moiety attached to the nitrogen of Y and is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an aryl C_1-C_6 alkylamino or arylheterocyclyl C_1-C_6 alkylamino;

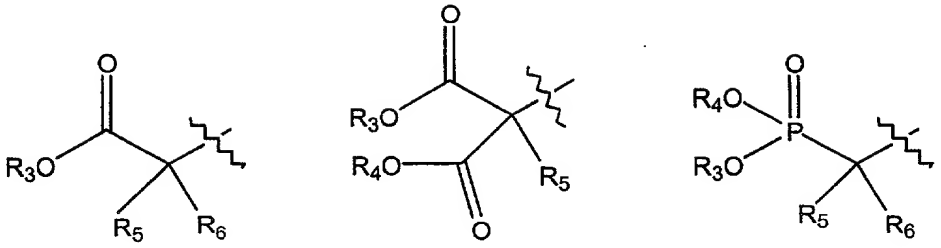
or a salt thereof. The compounds can be in D, L, or a mixed form thereof.

Preferred compounds of formula X include those wherein Y is of formula XI:



(XI),

wherein D has the formula XII, XIII, or XIV:



(XII) (XIII) (XIV)

wherein R_3 and R_4 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkaryl, and heteroaryl; and R_5 and R_6 may be the same or different and are selected from the group consisting of hydrogen, halo, hydroxy, amino, and C_1-C_6 alkoxy; and

E is selected from the group consisting of hydrogen, hydroxy, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, carboxyl, and C_1-C_6 alkylcarbonyl C_1-C_6 alkyl.

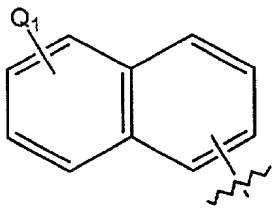
Particular examples of compounds of the present invention include compounds of formula X wherein D has the formula XII, XII, or XIII, and E is hydrogen, hydroxy, or carboxyl. In some embodiments, R_3, R_4, R_5, and R_6 are hydrogen. Certain embodiments include compounds wherein D has the formula XII, E is hydroxy or carboxyl, and R_3, R_5, and R_6 are hydrogen; and D has the formula XIII, E is hydrogen, R_3 and R_4 are hydrogen, and R_5 is hydrogen, hydroxy, alkyloxy, halo, keto, or alkyl, and preferably R_5 is hydrogen.

In certain embodiments, W is C_1-C_6 alkylcarbonyl, preferably C_1-C_3 alkylcarbonyl, for example, acetyl. In some embodiments, W is oxalyl or carboxymethylcarbonyl. In some other embodiments, W is tetrazolylcarbonyl, and preferably tetrazolylmethylcarbonyl. W can be an arylmethyloxycarbonyl, preferably, an aminophenylmethyloxycarbonyl, and more preferably 3-aminophenyl-1-methyloxycarbonyl in some embodiments. W can also be an aryloxycarbonyl, preferably a naphthyloxycarbonyl, and more preferably an aminonaphthyloxycarbonyl. An example of an aminonaphthyloxycarbonyl is 6-amino-1-naphthyloxycarbonyl.

The present invention also provides compounds of formula X wherein W is an arylmethyltetrazolylmethylcarbonyl, e.g., a phenylmethyl-

tetrazolylmethylcarbonyl. The present invention further provides compounds of formula X wherein W is an alkoxyphenylmethyltetrazolylmethylcarbonyl, e.g., a methoxyphenylmethyltetrazolylmethylcarbonyl.

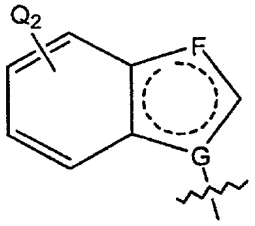
In embodiments of the compounds of formula X wherein Z is an aryl alkylamino, the aryl portion of Z has the formula:



wherein Q_1 is hydrogen or a substituent selected from the group consisting of hydroxyl, halo, C_1-C_6 alkyl, C_1-C_6 alkoxy, amino, and C_1-C_6 acylamino.

The aryl portion of Z is preferably attached to the alkylamino portion of Z at the aryl 1- or 2- position. A preferred compound of formula XI is one wherein Q_1 is hydrogen or methyl. A preferred compound of formula XI is one wherein Z is naphthylpropylamino.

In embodiments of the compounds of formula X wherein Z is aryl heterocyclyl alkylamino, the heterocyclyl portion of Z has the formula:



wherein Q_2 is hydrogen or a substituent selected from the group consisting of hydroxyl, halo, C_1-C_6 alkyl, C_1-C_6 alkoxy, amino, and C_1-C_6 acylamino, and F and G are independently selected from the group consisting of C, N, O, and S. A preferred F is C, and a preferred G is N. A preferred compound of formula X is one wherein Q_2 is

is hydrogen or methyl.

The number of amino acid segments or units in the compounds of formula X can be from 0 to 15. Compounds having smaller n values are preferred. For example, compounds wherein n is 1-10 are preferred; compounds wherein n is 1-3 are more preferred; and compounds wherein n is 2 are further preferred.

The compounds of formula X can include any suitable amino acid. For example, the amino acid can be selected from the group comprising, preferably consisting of, glycine, alanine, valine, norvaline, leucine, iso-leucine, norleucine, α-amino n-decanoic acid, serine, homoserine, threonine, methionine, cysteine, S-acetylaminomethyl-cysteine, proline, trans-3- and trans-4-hydroxyproline, phenylalanine, tyrosine, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, 4-aminocyclohexylglycine, 4-acylaminocyclhexylglycine, tryptophan, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aspartic acid, asparagine, aminomalonic acid, aminomalonic acid monoamide, glutamic acid, glutamine, histidine, arginine, lysine, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

Preferably, the amino acid is selected from the group consisting of leucine, iso-leucine, norleucine, cyclohexylalanine, cyclohexylglycine, 4-aminocyclohexylglycine, 4-acylaminocyclhexylglycine, aspartic acid, asparagine, glutamic acid, and glutamine.

It is further preferred that the compound of formula X includes a first amino acid (AA_1) attached to the phenylalanine moiety (Y) and asparagine attached to AA_1, wherein said AA_1 is selected from the group consisting of cyclohexylglycine, aspartic acid, glutamic acid, 4-aminocyclohexylglycine, 4-acylaminocyclohexylglycine, leucine, and isoleucine. A preferred compound of formula X is one wherein AA_1 is cyclohexylglycine.

Further examples of compounds of formula X are set forth in Fig. 2.

The compounds of formula I can be prepared by methods known to those skilled in the art. For example, the compounds can be prepared by the solid phase or solution phase peptide synthesis methods. Thus, the compounds can be prepared by reacting an amino acid or a peptide with the precursors of the present invention. Some embodiments of the synthetic method are illustrated in the Examples.

The present invention further provides a composition comprising a pharmaceutically acceptable carrier and an effective (e.g., therapeutically or prophylactically effective) amount of at least one of the compounds set forth above, particularly a compound of formula X. The present invention further provides a method of inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting a sample or substance containing an SH2 domain with a compound of formula X.

The present invention discloses the use of a compound of formula X in the manufacture of a medicament for the treatment of a condition that responds to the inhibition of phosphoprotein binding to an SH2 domain of a mammal. The present invention further teaches the use of a compound of formula X in medicine. The compounds of formula X find use as a Grb2-SH2 domain inhibitor.

The pharmaceutically acceptable (e.g., pharmacologically acceptable) carriers described herein, for example, vehicles, adjuvants, excipients, or diluents, are well-known to those who are skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active compounds and one which has no detrimental side effects or toxicity under the conditions of use.

The choice of carrier will be determined in part by the particular active agent, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of the pharmaceutical composition of the present invention. The following formulations for oral, aerosol, parenteral, subcutaneous, intravenous, intraarterial, intramuscular, interperitoneal, intrathecal, rectal, and vaginal administration are merely exemplary and are in no way limiting.

Formulations suitable for oral administration can comprise (a) liquid solutions, such as an effective amount of the compound dissolved in diluents,

such as water, saline, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations can include diluents, such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent, or emulsifying agent. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

The compounds of the present invention, alone or in combination with other suitable components, can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer.

Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compound can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile

liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, such as poly(ethyleneglycol) 400, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

The parenteral formulations will typically contain from about 0.5 to about 25% by weight of the active ingredient in solution. Suitable preservatives and buffers can be used in such formulations. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants. The quantity of surfactant in such formulations typically ranges from about 5 to about 15% by weight. Suitable surfactants include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The

parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

The compounds of the present invention may be made into injectable formulations. The requirements for effective pharmaceutical carriers for injectable compositions are well known to those of ordinary skill in the art. See Pharmaceutics and Pharmacy Practice, J.B. Lippincott Co., Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986).

Additionally, the compounds of the present invention may be made into suppositories by mixing with a variety of bases, such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

Suitable doses and dosage regimens can be determined by conventional range-finding techniques known to those of ordinary skill in the art. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired. In proper doses and with suitable administration of certain compounds, the present invention provides for a wide range of responses. Typically the dosages range from about 0.001 to about 1000 mg/kg body weight of the animal being treated/day. Preferred dosages range from about 0.01 to about 10 mg/kg body weight/day, and further preferred dosages range from about 0.01 to about 1 mg/kg body weight/day.

The compounds of formula X have the advantage that they are stable to or in presence of enzymes encountered during *in vivo* use. The compounds of formula X can find use in *in vitro* and *in vivo* applications. For example, the compounds can find use as molecular probes as well as in assays to identify,

human breast cancer are dependent upon activation of the *Ras* signaling pathways through activation of growth factor receptor as the means to achieve continuous cellular proliferation. For example, the cancer may involve overexpression of Her-2/neu. The cancer can be mediated through BCR-Abl or the expression of erbB-2 receptor. In cells transformed by p185 *erb*B-2 overexpression, therapeutic agents affecting Grb2 function at its SH2 domain may interrupt the flow of signal transduction to the ras pathway and thus result in reversal of the cancer phenotype.

The therapeutic treatment can include a chemotherapy, a radiation therapy, and/or a biological therapy. Examples of chemotherapy includes the use of cancer treatment agents such as alkylating agents, hormonal agents, antimetabolites, natural products, and miscellaneous agents. Particular examples of cancer treatment agents include paclitaxel, 5-fluoruracil, and doxorubicin. Examples of biological therapy includes the use of a protein such as an antibody (monoclonal or polyclonal) or a recombinant protein. An example of an antibody is herceptin, which is targeted for inhibiting the erbB-2 receptor. In embodiments, the enhancement of the therapeutic effect comprises blocking of a cell survival factor in the mammal and/or triggering, e.g., enhancing or speeding up, of cell apoptosis. The treatment can be carried out *in vivo* and/or *in vitro*.

The present invention further provides a method of inhibiting the MAP kinase activity in a mammal. MAP kinases function in a protein kinase cascade that plays a critical role in the regulation of cell growth and differentiation. MAP kinases are activated by a variety of signals including growth factors, cytokines and hormones through Grb2 and other signaling proteins. For example, the state of threonine and tyrosine phosphorylation of cellular MAP kinase is determined in MDA-453 cells treated with growth factor heregulin (HRG) using a polyclonal antibody specifically recognizing the phosphorylated threonine and tyrosine residues of MAP kinase. A dose-dependent inhibition of MAP kinase activity is observed in MDA-MB-453 cells. The IC_{50} value of MAP kinase inhibition is 12.5 μM for compound 126, which is in consistent with cell growth inhibition.

The Grb2 SH2 binding inhibitors are effective in inhibiting the association or binding of Grb2 with activated receptor PTKs. Interaction of

native Grb2 protein with phosphotyrosinylated proteins including receptor PTKs can be monitored by immunoprecipitating Grb2 and detecting the amount of phosphotyrosinylated proteins which are coprecipitated using anti-phosphotyrosine Western Blotting. For example, with compound 126, MDA-MB-453, BT-474 and SKBr3, show heavily phosphorylated proteins including a band corresponding to the overexpressed p185*erb*B-2. MDA-MB-468 and MDA-MB-231 cells show moderate to low level of phosphorylated protein at 170 kD corresponding to the overexpression of the EGFR. The NIH/3T3 fibroblasts engineered to express erbB-2 are also show growth inhibition.

The compounds of the present invention exert a cytostatic effect. For example, compound 126 inhibits MDA-MB-453 cells that have Grb2 activity through erbB-2 receptor overexpression. Tumor growth inhibition is also seen in MDA-453/M1 breast cancer xenografts in athymic mice with compound 126. The compounds of the present invention are free or substantially free of toxicity. For example, in female nude mice injected with human breast cancer cell line BT-474 cells, no systemic toxicity is observed with compound 126.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

This Example illustrates a method of preparing and characterizing certain compounds of formula I. The synthetic procedure is schematically illustrated in Fig. 1.

p-Tolyl-malonic acid di-tert-butyl ester (2). To the suspension of sodium hydride (60%, 1.2 g, 30 mmol) in 50 ml of anhydrous dioxane containing 3.5 ml of HMPA were added di-tert-butyl malonate (6.488 g, 30 mmol) and p-iodotoluene (**1**), the mixture was stirred at room temperature for 1 hour. To the resulting solution was added copper (I) bromide (5.163 g, 36 mmol, 1.2 equivalents), the mixture was heated at refluxing temperature. Then the reaction mixture was cooled to room temperature, 30 ml of saturated aqueous ammonium chloride solution was added to quench the reaction, and the product was extracted with ethyl acetate (50 ml X 3), washed with brine, dried over sodium sulfate. The solvent was evaporated, and the oily residue obtained was purified by chromatography to give p-tolyl-malonic acid di-tert-butyl ester 5.072 g (yield 55.2%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 7.275

(2H, d, J = 8.06 Hz), 7.161 (2H, d, J = 8.05 Hz), 4.397 (1H, s), 2.344 (3H, s), 1.471 (18H, s) ppm. FABMS ($^+$Ve), m/z 307 [MH^+], 251 [$MH^+ - C_4H_8$], 195 [$MH^+ - 2C_4H_8$]. Anal. calcd. for $C_{18}H_{26}O_4$: C, 70.6; H, 8.6. Found: C, 70.34; H, 8.62.

(4-Bromomethylphenyl)-malonic acid di-tert-butyl ester (3). p-Tolyl-malonic acid di-tert-butyl ester (5.695g, 16.6 mmol) was dissolved in 80 ml of CCl_4. To the solution was added N-bromosuccinimide (3.309 g, 16.6 mmol, 1 equivalents) and benzoyl peroxide (220 mg), the reaction mixture was refluxed under argon overnight; the reaction mixture was then cooled to room temperature, and the precipitate that had formed was filtered and washed with hexanes. The combined organic portions was dried, and the residue obtained was purified by chromatography to give (4-bromomethylphenyl)-malonic acid di-tert-butyl ester 4.2559 g (59.5% yield) as white solid. ^1H NMR ($CDCl_3$) δ: 7.379 (4H, s), 4.493 (2H, s), 4.435 (1H, s), 1.473 (18H, s) ppm. FABMS ($^+$Ve), m/z 387 [MH^+, ^{81}Br], 385 [MH^+, ^{79}Br], 331 [$MH^+ - C_4H_8$, ^{81}Br], 329 [$MH^+ - C_4H_8$, ^{79}Br], 275 [$MH^+ - 2C_4H_8$, ^{81}Br], 273 [$MH^+ - 2C_4H_8$, ^{79}Br]. Anal. calcd. for $C_{18}H_{25}BrO_4$: C, 56.1; H,6.5; Br, 20.7. Found: C, 55.52; H, 6.38; Br, 21.85.

Benzyl (3S,5S,6R)-3-[4-(di-tert-butoxycarbonyl-methyl)phenylmethyl]- (-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate (4). To a solution of benzyl (2R,3S)-(-)-6-oxo-2,3-diphenyl-4-morpholine-carboxylate (2.688 g, 6.94 mmol) in anhydrous tetrahydrofuran (60 ml) and HMPA (4.6 ml) cooled to -78°C under an argon atmosphere was added lithium bis(trimethylsilyl)amide (1.0M solution in hexanes, 7.29 ml, 7.29 mmol, 1.05 equivalents). The reaction mixture was stirred at -78°C for 1 hour. A solution of 2-(4-bromomethylphenyl)-malonic acid di-tert-butyl ester (2.6755 g, 6.94 mmol) in THF was added slowly at -78°C via a syringe, and the mixture was first stirred at -78°C for 2 hours and then the temperature was raised to room temperature, and the mixture was stirred overnight. The mixture was then quenched with aqueous NH_4Cl (10 ml) and diluted with 35 ml of water. The mixture was extracted with ethyl acetate repeatedly, and the combined organic extracts were washed successively with water, aqueous

NH_4Cl, and brine, dried over Na_2SO_4. Concentration and purification by silica gel chromatography (hexanes-ethyl acetate, from 6:1 to 3:1) gave benzyl (3S,5S,6R)-3-[4-(di-tert-butoxycarbonyl-methyl)phenylmethyl]-(-)-6-oxo-5,6-diphenyl-4-morpholine carboxylate as a white solid (1.88 g, 39.2% yield). ^1H NMR ($CDCl_3$) (two conformers were observed in a ratio of 5:7 at 23°C) δ: 7.462~7.354 (3H, m, overlapping), 7.285~7.046 (10 H, m, overlapping), 6.914~6.720 (4H, m, overlapping), 6.546 (2H, m, overlapping); major conformer: 5.374 (1H, d, J = 2.69 Hz, -PhCHOOC-), 5.172~5.045 (3H, m, overlapping, -OOCCH-N, -PhCHN-, OCH_2Ph), 4.960 (1H, d, J = 13.19 Hz, OCH_2Ph), 4.655 [1H, s, $(tBuOOC)_2CH$-], 3.606~3.518 (1H, dd, J = 8.06, 13.43 Hz, -CH_2-CHNCOO), 3.455~3.358 (1H, m, -CH_2-CHNCOO), 1.420 (9H, s), 1.394 (9H, s) ppm; minor confomer: 5.708 (1H, d, J = 2.20 Hz, -PhCHOOC-), 5.172~5.045 (4H, m, overlapping, -OOCCH-N, -PhCHN-, OCH_2Ph), 4.626 [1H, s, $(tBuOOC)_2CH$-], 3.606~3.350 (2H, m, overlapping, -CH_2-CHNCOO), 1.421 (9H, s), 1.397 (9H, s) ppm; FABMS ($^+$ve), m/z 580.5 [MH^+ -2 C_4H_8], 536.5 [MH^+ - 2C_4H_8 - CO_2], 492.5 [MH^+ - 2C_4H_8 - 2CO_2]. Anal. calcd. for $C_{42}H_{45}NO_8$: C, 72.9; H, 6.6; N, 2.0. Found: C, 72.62; H, 6.69; N, 1.85.

4-(Di-tert-butoxycarbonyl-methyl)-L-phenylalanine (5). Benzyl (3S,5S,6R)-3-[4-(di-tert-butoxycarbonyl-methyl)phenylmethyl]-(-)-6-oxo-5,6-diphenyl-4-morpholine carboxylate (1.78 g, 2.57 mmol) was dissolved in THF-EtOH mixture (1:1, 15 ml, 2 drops of AcOH was added to promote the reaction) and hydrogenated over Pd black (200 mg) under high pressure (45 psi ~ 20 psi or 310 kPa to about 138 kPa) at room temperature (24 hours). The mixture was filtered off and the solid was washed with MeOH. The combined organics were concentrated to give a white sticky solid. The solid was washed thoroughly with ether to remove 1,2-diphenylethane and dried under vacuum to provide 4-(di-tert-butoxycarbonyl-methyl)-L-phenylalanine as a white powder (842 mg, 86.3%). ^1H NMR (DMSO) δ: 7.251 (4H, s), 4.590 (1H, s), 3.422~3.316 (3H, m, OOCCH-N, -NH_2), 3.17~3.01 (1H, m, -CH_2-CHNCOO), 2.943~2.785 (1H, m, -CH_2-CHNCO), 1.416 (18H, S) ppm; FABMS ($^+$Ve), m/z 268 [MH^+ -2 C_4H_8]. Anal. calcd. for $C_{20}H_{29}NO_6$: C, 63.3; H, 7.7; N, 3.7. Found: C, 63.26; H, 7.82; N, 3.52.

N-Fmoc-4-(di-tert-butoxycarbonyl-methyl)-L-phenylalanine (6). A mixture of 4-(di-tert-butoxycarbonyl-methyl)-L-phenylalanine (818 mg, 2.16 mmol), Fmoc-OSu (727 mg, 2.16 mmol) and $NaHCO_3$ (906 mg, 10.8 mmol, 5 equivalents) in 48 ml of dioxane-water (1:1) was stirred at room temperature overnight. The reaction mixture was then cooled to 0°C and acidified with 180 ml of 0.2 M HCl. The reaction product was extracted with ethyl acetate (30 ml x 3), and the combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated . The crude product was purified by silica gel chromatography ($CDCl_3$-EtOAc-MeOH) to provide N-Fmoc-4-(di-tert-butoxycarbonyl-methyl)-L-phenylalanine as a white solid (650 mg, 50.7%).^{1}H NMR (DMSO) δ: 12.704 (1H, s, br), 7.786 (2H, d, J = 7.32 Hz), 7.760 (1H, d, J = 8.54 Hz), 7.658 (2H, t, J = 7.81 Hz), 7.435~7.200 (4H, m), 7.254 (4H, s), 4.586 (1H, s), 4.274~4.081 (4H, m), 3.111~3.040 (1H, dd, J = 4.40, 13.67 Hz), 2.913~2.815 (1H, dd, J = 10.75, 13.92 Hz), 1.390 (1H, s), 1.380 (1H, s). FABMS ($^+$Ve), m/z 602 [MH^+], 546 [$MH^+ - C_4H_8$], 490 [$MH^+ - 2\ C_4H_8$]. Anal. calcd. for $C_{20}H_{29}NO_6$: C, 66.90; H, 6.5; N, 2.3. Found: C, 69.40; H, 6.67; N, 2.24.

EXAMPLE 2

This Example illustrates a method of preparing embodiments of formula X. The reactions are schematically depicted in Figs. 4-14.

Compound 8. To the solution of compound **7** (45.5 mg, 0.1 mmol) in anhydrous DMF (2 ml) was added an active ester solution formed by reacting N-Fmoc-4-(di-tert-butoxycarbonyl-methyl)-L-phenylalanine (60.1 mg, 0.1 mmol), HOBt.H_2O (13.5 mg, 0.1 mmol) and DIPCDI (15.6 µl, 0.1 mmol) in anhydrous DMF (2 ml) at room temperature (10 min.). The reaction mixture was then stirred at room temperature overnight. The solvent was removed under high vacuum and the residue obtained was purified by silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to provide the desired product, compound **8**, as a white foam (100% yield). ^{1}H NMR ($CDCl_3$) δ: 8.01 (2H, m), 7.81~7.71 (6H, m), 7.50~6.95 (12H, m), 6.58 (1H, s), 5.56 (3H, m), 4.69 (1H, m), 4.55~4.40 (1H, m), 4.40 (1H, s), 4.31 (2H, d, J = 6.84 Hz), 4.09

(1H, m), 3.34 (1H, m), 3.12~2.88 (5 H, m), 2.63 (1H, dd, J = 4.4, 15.1 Hz), 2.05~1.11 (12H, m), 1.46 (18H, s) ppm. FABMS ($^+$Ve), m/z 1009 [MH^+].

Compound 9. To the solution of compound **8** (0.05 mmol) in anhydrous acetonitrile (2 ml) was added piperidine (40 µl, 0.4 mmol, 8 equivalents) and the solution was stirred at room temperature for 3 hr. At the end, the solvent and the excess piperidine were removed under high vacuum. The residue obtained was dissolved in anhydrous DMF (2 ml). To the resulting solution was added diisopropylethylamine (13 µl, 0.075 mmol, 1.5 equivalents) followed by the addition of tert-butyl oxalyl chloride (9.5 µl, 0.075 mmol, 1.5 equivalents). The solution was stirred at room temperature overnight. The mixture was concentrated and purified by on silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to provide product 22.8 mg (50% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ: 8.02 (2H, m), 7.81~7.21 (13H, m), 6.69 (1H, s),6.38 (1H, s), 4.67 (2H, m), 4.40 (1H, s), 3.43~2.88 (7 H, m), 2.56 (1H, dd, J = 4.88, 14.89 Hz), 2.05~0.94 (12H, m), 1.464 (18H, s), 1.457 (9H, s) ppm. FABMS ($^+$Ve), m/z 914 [MH^+], HR-FABMS calcd for $C_{50}H_{67}N_5O_{11}$ m/z 913.4837 (M+), 914.4915 (MH+), found 913.4837, 914.4915.

Compound 10. To the solution of compound **8** (0.05 mmol) in anhydrous acetonitrile (2 ml) was added piperidine (40 µl, 0.4 mmol, 8 equivalents) and the solution was stirred at room temperature for 3 hr. At the end, the solvent and the excess piperidine were removed under high vacuum. The residue was taken up in anhydrous acetonitrile (3.0 ml) and treated with N-acetylimidazole (55 mg, 0,5 mmol, 10 equivalents). The solution was stirred at room temperature overnight. The solvent was removed and the residue was purified by on silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to provide product 38.9 mg of product (94% yield) as white foam. ^{1}H NMR ($CDCl_3$) δ: 8.03 (2H, m), 7.81 (1H, m), 7.63 (3H, m), 7.46~7.05 (9H, m), 6.65 (2H, m), 4.67 (2H, m), 4.40 (1H, s), 3.34 (2H, m), 3.12~2.89 (5 H, m), 2.55 (1H, dd, J = 4.60, 15.1 Hz), 1.806 (3H, s), 2.04~1.15 (12H, m), 1.46 (18H, s) ppm. FABMS ($^+$Ve), m/z 828 [MH^+], 772 [$MH^+ - C_4H_8$], HR-FABMS calcd for $C_{46}H_{61}N_5O_9$ m/z 827.4469 (M+), 828.4548(MH+), found 827.4469, 828.4548.

Compound 11. A solution of compound **10** (22.3 mg, 0.0244 mmol) in TFA-H_2O-Triethylsilane (1.85 ml - 0.1 ml -50 µl) was stirred at room temperature for 1 hr, then the solvents were evaporated under high vacuum. 5 ml of water were added and the mixture was taken to dryness again to remove the remaining TFA. This procedure was repeated two times. The crude products obtained were dissolved in MeCN: H_2O (1:1, plus 0.1% TFA, 6 ml), filtered and purified by HPLC, using an Advantage C_{18} column (250 mm x 20 mm dia.) with a flow rate: 10 ml/min and a linear gradient from 5% B to 100% B over 40 min.; solvent A: 0.1% aqueous TFA; solvent B: 0.1% TFA in MeCN. Compound **11** was obtained as a white solid (9.4 mg, 52%). ^{1}H NMR (DMSO) δ: 8.82 (1H, s), 8.08 (1H, m), 8.00 (1H, d, J = 7.33 Hz), 7.89 (1H, m), 7.74 (1H, m), 7.53~7.20 (10H, m), 6.92 (1H, s), 4.73 (1H, m), 4.60 (1H, s), 4.36 (1H, m), 3.25~2.93 (6H, m), 2.69 (1H, dd, J = 6.75, 15.4 Hz), 2.52 (1H, m), 2.08~1.12 (12H, m) ppm. FABMS ($^-$Ve), m/z 744 [M-H], 700 [M-H- CO_2], HR-FABMS calcd for $C_{38}H_{43}N_5O_{11}$ m/z 745.2959 (M), 744.2881(M-H), found 745.2959, 744.2881.

Compound 12. A solution of **10** (38.4 mg, 0.047 mmol) in TFA-H_2O-Triethylsilane (1.85 ml - 0.1 ml -50 µl) was stirred at room temperature for 1 hr. The solvents were evaporated under high vacuum. 5 ml of water were added and the mixture was taken to dryness again to remove the remained TFA. This procedure was repeated twice. The crude product obtained was dissolved in MeCN : H_2O (1 : 1, plus 0.1% TFA, 10 ml), filtered and purified by HPLC, using an Advantage C_{18} column (250 mm x 20 mm dia.) with a flow rate: 10 ml/min and a linear gradient from 5% B to 100% B over 40 min.; solvent A: 0.1% aqueous TFA; solvent B: 0.1% TFA in MeCN. Compound **12** was obtained as a white solid (24.5 mg, 73%). ^{1}H NMR (DMSO) δ: 8.25 (2H, m), 8.08 (1H, m), 7.99 (1H, m), 7.90 (1H, m), 7.74 (1H, m0, 7.51~7.18 (10H, m), 6.90 (1H, s), 4.65 (1H, m), 4.60 (1H, s), 4.36 (1H, m), 3.35~2.98 (5H, m), 2.82~2.56 (3H, m), 1.78 (3H, s), 2.08~1.12 (12H, m) ppm. FABMS ($^-$Ve), m/z 714 [M-H], 670 [M-H- CO_2], 626 [M-H- $2CO_2$], HR-FABMS calcd for $C_{38}H_{45}N_5O_9$ m/z 715.3217 (M), 714.3139(M-H), found 745.2959, 744.2881.

Compound 13. To the solution of compound **7** (63.6 mg, 0.14 mmol) in anhydrous DMF (2 ml) was added an active ester solution formed by N-Fmoc-[3-acetoxyl-4-(tert-butoxycarbonyl)methyl]-L-phenylalanine (78.3 mg, 0.14 mmol), HOBt.H_2O (18.9 mg, 0.14 mmol) and DIPCDI (21.8 µl, 0.14 mmol) in anhydrous DMF (2 ml) at room temperature (10 min.). The combined reaction mixture was then stirred at room temperature overnight. The solvent was removed under high vacuum and the residue obtained was purified by silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to compound **13** (123.7 mg, 91.4% yield), as a white foam. ^{1}H NMR ($CDCl_3$) δ: 8.03 (2H, m), 7.82~7.23 (17 H, m), 7.17 (1H, d, J = 7.81 Hz), 6.95 (2H, m), 6.79 (1H, s), 6.26 (1H, s), 4.69 (1H, m), 4.46~4.34 (3H, m), 4.14 (1H, m), 3.42 (2H, s), 3.41~3.32 (2H, m), 3.14~2.94 (4H, m), 2.64~2.45 (2H, m), 2.27 (3H, s), 2.06~1.11 (12H, m) ppm, 1.42 (9H, s). FABMS ($^+$Ve), m/z 966 [MH^+], 949 [MH^+-NH_3].

Compound 14. To the solution of compound **13** (61.4 mg, 0.064 mmol) in anhydrous acetonitrile (2.5 ml) was added piperidine (50 µl, 0.51 mmol, 8 equivalents) and the solution was stirred at room temperature for 3 hr. The solvent and the excess piperidine were removed under high vacuum. The residue obtained was dissolved in anhydrous DMF (2 ml), to the solution was added diisopropylethylamine (22 µl, 0.126 mmol, 2 equivalents) followed by the addition of tert-butyl oxalyl chloride (16 µl, 0.126 mmol, 2 equivalents). The solution was stirred at room temperature overnight. The solution was concentrated and purified by silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to provide compound **14**, 51 mg (92% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ: 8.04~7.00 (12H, m), 6.79 (1H, s), 6.70 (1H, d, J = 1.22 Hz), 6.46 (2H, m), 4.80~4.60 (2H, m), 3.53 (2H, s), 3.45~2.76 (7H, m), 2.56 (1H, dd, J = 4.88, 15.13 Hz), 2.28 (3H, s), 2.18~1.15 (12H, m), 1.50 (9H, s), 1.46 (9H, s). FABMS ($^+$Ve), m/z 872 [MH^+].

Compound 15. To a solution of compound **13** (61.4 mg, 0.064 mmol) in anhydrous acetonitrile (2.5 ml) was added piperidine (50 µl, 0.51 mmol, 8 equivalents) and the solution was stirred at room temperature for 3 hr. The

solvent and the excess piperidine were removed under high vacuum. The residue was taken up in anhydrous acetonitrile (3.0 ml) and treated with N-acetylimidazole (69.3 mg, 0.63 mmol, 10 equivalents). The solution was stirred at room temperature overnight. The solvent was removed under vacuum at 30°C and the residue was purified by silica gel chromatography ($CHCl_3$-EtOAc-MeOH mixture) to provide product , compound **15**, 41 mg (82% yield) as a white foam. ^1H NMR ($CDCl_3$) δ: 8.033 (2H, m), 7.81 (1H, m), 7.68 (1H, m), 7.57~7.33 (6H, m), 7.26~6.95 (5H, m), 6.42 (1H, s),6.27 (1H, d, J = 7.3 Hz), 4.66 (2H, m), 3.42 (2H, s), 3.37~3.31 (2H, m), 3.14~2.87 (5H, m), 2.55 (1H, dd, J = 4.88, 15.13 Hz), 2.28 (3H, s), 1.83 (3H, s), 2.07~1.12 (12H, m), 1.42 (9H, s). FABMS ($^+$Ve), m/z 786 [MH^+], 769 [MH^+-NH_3].

Compound 16. A solution of compound **14** (51 mg, 0.058 mmol) in 3 ml of benzene containing phenethylamine (100 µl, 0.6 mmol, 10 equivalents) was stirred at room temperature for 2.5 hr and benzene was evaporated. The residue was treated with TFA-water-triethylsilane mixture (3.7 ml: 0.2 ml : 0.1 ml) for 1 hr and then taken to dryness under high vacuum at room temperature. 5 ml of water were added to the mixture, and the mixture was taken to dryness again to remove the remaining TFA. This procedure was repeated twice. The crude product obtained was dissolved in MeCN:H_2O (1:1, plus 0.1% TFA, 10 ml), filtered and purified by HPLC, using an Advantage C_{18} column (250 mm x 20 mm dia.) with a flow rate: 10 ml/min and a linear gradient from 5% B to 100% B over 40 min.; solvent A: 0.1% aqueous TFA; solvent B: 0.1% TFA in MeCN. Compound **16** was obtained as a white solid (17.7 mg, 42.5%). ^1H NMR (DMSO) δ: 9.33 (1H, s, -OH), 8.83 (1H, m), 8.46 (1H, d, j = 7.81 Hz), 8.32 (1H, s), 8.09 (1H, m), 7.92 (2H, m), 7.74 (1H, m), 7.57~7.45 (6H, m), 7.28~7.13 (5H, m), 6.96 (2H, m), 6.69 (1H, s), 6.59 (1H, dd, J = 7.82 Hz), 4.67 (1H, m), 4.40 (1H, m), 3.40 (2H, s), 3.31 (2H, q, J = 6.35 Hz), 3.20~3.01 (5H, m), 2.87 (1H, dd, J = 10.50, 15.20Hz), 2.76~2.68 (3H, m), 2.65~2.33 (1H, dd, J = 6.34, 14.90 Hz), 1.96~1.18 (12H, m). FABMS ($^-$Ve), m/z 819.5 [M-H], 775.4 [M- H-CO_2]. HR-FABMS calcd for $C_{37}H_{43}N_5O_{10}$ m/z 716.2932 (M-H).

Compound 17. A solution of compound **15** (41 mg, 0.052 mmol) in 3 ml of benzene containing phenethylamine (100 µl, 0.6 mmol, 10 equivalents) was stirred at room temperature for 2.5 hr, and at the end, the benzene was evaporated. The residue obtained was treated with TFA-water-triethylsilane mixture (3.7 ml : 0.2 ml : 0.1 ml) for 1 hr and then taken to dryness under high vacuum at room temperature. 4 ml of water were added and the mixture was taken to dryness again to remove the remained TFA. This procedure was repeated twice. The crude product obtained was dissolved in MeCN:H_2O (1 : 1, plus 0.1% TFA, 10 ml), filtered and purified by HPLC, using an Advantage C_{18} column (250 mm x 20 mm dia.) with a flow rate: 10 ml/min and a linear gradient from 5% B to 100% B over 40 min.; solvent A: 0.1% aqueous TFA; solvent B: 0.1% TFA in MeCN. Product 106C-96 was obtained as a white solid (24.7.7 mg, 69%). ^{1}H NMR (DMSO) δ:9.29 (1H, s, br, -OH), 8.20 (2H, m), 8.08 (1H, m), 7.96 (1H, d, J = 7.82 Hz), 7.90~7.87 (1H, m), 7.74 (1H, m), 7.53~7.33 (6H, m), 6.96 (1H, d, J = 7.81 Hz), 6.90 (1H, s), 6.70 (1H, s), 6.64 (1H, d, J = 7.81 Hz), 4.61 (1H, m), , 4.36 (1H, m), 3.41 (2H, s), 3.17 (2H, m), 3.07~-2.93 (3H, m), 2.75~2.56 (3H, m), 1.78 (3H, s), 1.98~1.14 (12H, m). FABMS ($^+$Ve), m/z 710 [M+ Na^+], 688 [MH^+], 671 (MH^+-NH_3). HR-FABMS calcd for $C_{37}H_{46}N_5O_8$ (MH^+) m/z 688.3346.

Additional examples of preparing compounds (**18**-**20**) of formula X are set forth in Fig. 3.

EXAMPLE 3

This Example illustrates a method of preparing some compounds of formula I. The reactions involved are schematically illustrated in Fig. 14.

2-Carboxymethyl-5-methyl-benzoic acid was prepared according to the published methods (J. Org. Chem., 4689 (1962)).

2-Tert-butoxycarbonylmethyl-5-methyl benzoic acid tert-butyl ester (21). To a suspension of 2-carboxymethyl-5-methyl-benzoic acid (2.544 g, 13.1 mmol) in anhydrous dichloromethane (70 ml) held at 0°C was added tert-butyl 2,2,2-trichloroacetimidate (11.47 mg, 52.4 mmol, 2 equivalents) in cyclohexane (85 ml). BBr_3 (0.52 ml) was then added, and the reaction mixture was raised to room temperature and stirred for 16 hr. Solid $NaHCO_3$

was added to quench the reaction, the solid precipitate was filtered off and washed with ether. The combined organic washing was evaporated to dryness, and the residue obtained was purified by silica gel chromatography to obtain 2-tert-butoxycarbonylmethyl-5-methyl benzoic acid tert-butyl ester 3.345 g (83.3%) as an oil. ^{1}H NMR ($CDCl_3$): 7.718 (1H, s), 7.227 (1H, d, J = 7.57 Hz), 3.910 (2H, s), 2.362 (3H, s), 1.584 (9H, s), 1.443 (9H, s). FABMS ($^+$Ve), m/z 307 [MH^+], 251 [$MH^+ - C_4H_8$], 195 [$MH^+ - 2C_4H_8$]. Anal. calcd. for $C_{18}H_{26}O_4$: C, 70.6; H, 8.6. Found: C, 70.82; H, 8.53.

2-Tert-butoxycarbonylmethyl-5-bromomethyl benzoic acid tert-butyl ester (22). 2-Tert-butoxycarbonylmethyl-5-methyl benzoic acid tert-butyl ester (4.751, 15.5 mmol) was dissolved in 75 ml of CCl_4. To the solution was added N-bromosuccinimide (2.90 g, 16.3 mmol, 1.05 equivalents) and benzoyl peroxide (180 mg). The reaction mixture was refluxed under an argon atmosphere overnight; the reaction mixture was then cooled to room temperature, and the solid precipitate was filtered off, and washed with hexanes, the combined organic was taken to dryness and the residue was purified by chromatography to obtain 2-tert-butoxycarbonylmethyl-5-bromomethyl benzoic acid tert-butyl ester 2.286 g (38.3% yield) as an oil. ^{1}H NMR ($CDCl_3$): 7.920 (1H, d, J = 2.2 Hz), 7.460 (1H, dd, J = 1.95, 7.81 Hz), 7.193 (1H, d, J = 7.81 Hz), 4.500 (2H, s), 3.953 (2H, s), 1.590 (9H, s), 1.444 (9H, s). FABMS ($^+$Ve), m/z 387 [MH^+, ^{81}Br], 385 [MH^+, ^{79}Br], 331 [$MH^+ - C_4H_8$, ^{81}Br], 329 [$MH^+ - C_4H_8$, ^{79}Br], 275 [$MH^+ - 2C_4H_8$, ^{81}Br], 273 [$MH^+ - 2C_4H_8$, ^{79}Br]. Anal. calcd. for $C_{18}H_{25}BrO_4$: C, 56.1; H, 6.5; Br, 20.7. Found: C, 56.27; H, 6.60; Br, 20.75.

Benzyl (3S,5S,6R)-3-{[3-tert-butoxycarbonyl-4-(tert-butoxycarbonyl-methyl)phenyl]-methyl}-(-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate (23). To a solution of benzyl (2R,3S)-(-)-6-oxo-2,3-diphenyl-4-morpholine-carboxylate (2.298 g, 5.93 mmol) in anhydrous tetrahydrofuran (50 ml) and HMPA (4.0 ml) cooled to -78°C under an argon atmosphere was added lithium bis(trimethylsilyl)amide (1.0M solution in hexanes, 6.23 ml, 6.23 mmol, 1.05 equivalents). The reaction mixture was stirred at -78°C for 1 hour. A solution of 2-tert-butoxycarbonylmethyl-5-bromomethyl benzoic acid tert-butyl ester (2.286 g, 5.93 mmol) in THF (10 ml) was added slowly at -78°C via a syringe, and the mixture was stirred at -78°C for 2 hours. The temperature was then raised to room temperature, and stirred overnight. The reaction mixture was quenched with aqueous NH_4Cl (10 ml) and diluted with 35 ml of water. The mixture was extracted with ethyl acetate (50 ml x 3), and the combined organics were washed successively with water, aqueous NH_4Cl, and brine, dried over Na_2SO_4. Concentration and purification by silica gel chromatography (hexanes-ethyl acetate, from 6:1 to 3:1) gave benzyl (3S,5S,6R)-3-{[3-tert-butoxycarbonyl-4-(tert-butoxycarbonylmethyl)phenyl]-methyl}-(-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate as a white foam (2.8188 g, 68.7% yield). ^1H NMR ($CDCl_3$) (two conformers were observed in a ratio of 2 : 5 at 23°C) δ: 7.834 (1H, d, J = 1.96 Hz), 7.417~7.317 (2H, m, overlapping), 7.230~7.046 (10 H, m, overlapping), 6.757~6.589 (4H, m, overlapping), 6.502 (1H, d, J = 7.3 Hz, overlapping); major conformer: 5.352 (1H, dd, J = 2.93, 6.59 Hz, -OOCCH-N), 5.113 (1H, d, J = 12.2 Hz, OCH_2Ph), 4.931 (1H, d, J = 12.20 Hz, OCH_2Ph), 4.881 (1H, d, J = 2.93 Hz, COOCHPh-), 4.520 (1H, d, J = 2.93 Hz, PhCHN-), 4.037 (1H, d, J = 16.84 Hz, -$CH_2COOtBu$), 3.874 (1H, d, J = 17.09 Hz, $NCH_2COOtBu$), 3.743 (1H, dd, J = 6.84, 13.92 HZ), 3.440 (1H, dd, J = 2.93, 13.91 HZ), 1.523 (9H, s), 1.452 (9H, s); minor confomer: 7.747 (1H, s), 5.708 (1H, d, J = 2.20 Hz, -PhCHOOC-), 7.417~7.317 (2H, m, overlapping), 7.230~7.046 (10 H, m, overlapping), 6.757~6.589 (4H, m, overlapping), 6.502 (1H, d, J = 7.3 Hz, overlapping), 5.248 (1H, dd, J = 3.66, 7.72 Hz, -OOCCH-N), 5.287 (1H, d, J = 11.97 Hz, OCH_2Ph), 5.117 (1H, d, J = 12.5 Hz, OCH_2Ph), 5.047 (1H, d, J = 2.93 Hz, COOCHPh-), 4.811 (1H, d, J = 2.93 hz, NCHPh-), 3.986 (1H, J = 16.60 Hz, -$CH_2COOtBu$), 3.896 (1h, d, J - 17.09 Hz, -$CH_2COOtBu$), 3.512 (1H,

dd, J = 7.32, 13.91 Hz), 3.383 (1H, dd, J = 3.42, 13.91 Hz), 1.557 (9H, s), 1.542 (9H, s); FABMS (⁻Ve) m/z 690 [M- H], 634 [M-H-C_4H_8]. Anal. calcd. for $C_{42}H_{45}NO_8$: C, 72.9; H, 6.6; N, 2.0. Found: C, 73.07; H, 6.70; N, 1.95.

(3-tert-butoxycarbonyl-4-tert-butoxycarbonylmethyl)-L-phenylalanine (24). Benzyl (3S,5S,6R)-3-{[3-tert-butoxycarbonyl-4-(tert-butoxycarbonyl-methyl)phenyl]-methyl}-(-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate (2.7388 g, 3.96 mmol) was dissolved in THF-EtOH mixture (2:1, 24 ml) and hydrogenated over Pd black (250 mg) under high pressure (45 psi ~ 20 psi or 310 kPa to about 138 kPa) at room temperature (24 hours). At the end, the palladium black was filtered off and washed with MeOH. The combined organic was concentrated to give a white sticky solid. This crude product was washed thoroughly with ether to remove 1,2-diphenylethane and dried under vacuum to obtain (3-tert-butoxycarbonyl-4-tert-butoxycarbonylmethyl)-L-phenylalanine as a white powder (851 mg, 56.6%). ^{1}H NMR (DMSO) δ: 7.700 (1H, s), 7.376 (1H, d, J = 76.6 Hz), 7.206 (1H, d, J = 6.6 hz), 3.876 (2H, s), 3.45~3.25 (2H, m), 3.154 (1H, dd, J = 4.15, 14.16 Hz), 2.851 (1H, dd, J = 7.56, 14.16 Hz), 1.514 (9H, s), 1.386 (9H, s) ppm; FABMS (⁺Ve) m/z 380 [MH^+], 324 [MH^+ -C_4H_8], 268 [MH^+ - $2C_4H_8$]. Anal. calcd. for $C_{20}H_{29}NO_6$: C, 63.3; H, 7.7; N, 3.7. Found: C, 63.53; H, 7.72; N, 3.68.

N-Fmoc-(3-tert-butoxycarbonyl-4-tert-butoxycarbonylmethyl)-L-phenylalanine (25). A mixture of (3-tert-butoxycarbonyl-4-tert-butoxycarbonylmethyl)-L-phenylalanine (830 mg, 2.24 mmol), Fmoc-OSu (754 mg, 2.24 mmol) and $NaHCO_3$ (1.5 mg, 17.9 mmol, 8 equivalents) in 50 ml of dioxane-water (1:1) was stirred at room temperature overnight, and the reaction mixture was cooled to 0°C and acidified with 180 ml of 0.2 M HCl. The reaction product was extracted with ethyl acetate (50 ml x 3), the combined organics were washed with brine, dried (Na_2SO_4) and concentrated. The crude product was purified by silica gel flash chromatography ($CDCl_3$-EtOAc-MeOH) to provide N-Fmoc-(3-tert-butoxycarbonyl-4-tert-butoxycarbonylmethyl)-L-phenylalanine as a foam (1.032, 78.4%). ^{1}H NMR (DMSO): 12.729 (1H, s, br), 7.874 (2H, d, J = 7.57 Hz),7.804 (1H, d, J =

8.55 Hz), 7.754 (1H, s), 7.627 (2H, m), 7.450-7.194 (6H, m), 4.30-4.10 (4H, m), 3.861 (2H, s), 3.119 (1H, dd, J = ,4.39, 14.16 Hz), 2.910 (1H, dd, J = 11.48, 13.18 Hz), 1.489 (9H, s), 1.372 (9H, s). FABMS ($^+$Ve), m/z 602 [MH^+], 490 [MH^+ -2 C_4H_8]. HR-FABMS calcd for $C_{20}H_{29}NO_6$: 601.2676.

EXAMPLE 4

This Example illustrates a method of preparing some other embodiments of formula I. The reactions involved are schematically illustrated in Fig. 15.

[4-Methyl-2-(phenylmethoxy)phenyl]acetic acid was prepared according to the published methods (G. W. Rewcastle et al., J. Med. Chem., 32, 793-799 (1989)).

[4-Methyl-2-(phenylmethoxy)phenyl]acetic acid tert-butyl ester (26). To a solution of [4-methyl -2-(phenylmethoxy)phenyl]acetic acid (10.25 g, 40 mmol) in toluene (70 ml containing 2 drops of DMF) was added oxalyl chloride (4.0 ml, 46 mmol, 1.15 equivalents) dropwise, the mixture was stirred at room temperature for 5 hr then 44 ml of tert-butanol was added, the resulting solution was stirred at room temperature for 20 hr. The solvents were evaporated and the residue obtained was purified by chromatography on silica gel (EtOAc-hexanes, 1 : 40 to 1 : 25) to provide the desired product [4-methyl-2-(phenylmethoxy)-phenyl]acetic acid tert-butyl ester as an oil (11.70g, 92.8% yield). ^{1}H NMR ($CDCl_3$) δ: 7.521~7.364 (5H, m), 7.147 (1H, d, J = 7.81 Hz), 6.810 (2H, m), 5.118 (2H, s), 3.629 (2H, S), 2.391 (3H, s), 1.461 (9H, s) ppm.

(2-Hydroxy-4-methylphenyl)acetic acid tert-butyl ester (27). To a solution of [4-methyl -2-(phenylmethoxy)phenyl]acetic acid tert-butyl ester (10.67 g, 34.2 mmol) in 125 ml of ethanol was added palladium black (200 mg), the mixture was hydrogenated (using a hydrogen balloon) at 30°C for 10 hr. After the starting material disappeared completely (detected by TLC), the solid was filtered off, and the solvent was evaporated to give a crude product of (2-hydroxy-4-methylphenyl)acetic acid tert-butyl ester, 7.32 g (96%) as white solid. ^{1}H NMR ($CDCl_3$) δ: 7.931 (1H, s, br), 6.955 (1H, d, J =

7.56 Hz), 6.799 (1H, s), 6.688 (1H, d, J = 7.81 Hz), 3.551(2H, S), 2.299 (3H, s), 1.472 (9H, s) ppm.

(2-Acetoxyl-4-methylphenyl)acetic acid tert-butyl ester (28). To a solution of (2-hydroxy-4-methylphenyl)acetic acid tert-butyl ester (4.45 g, 20 mmol) in pyridine (6.5 ml) was added acetyl anhydride (5.1 g, 50 mmol) at room temperature, and the resulting solution was stirred at room temperature overnight. The pyridine and the remaining acetyl anhydride were removed by applying high vacuum at 30°C. 20 ml of toluene were added to the residue, and the toluene was evaporated to remove the residual pyridine. The crude product obtained was purified by chromatography on silica gel to provide product (2-acetoxyl-4-methylphenyl)acetic acid tert-butyl ester (4,95 g, 94%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 7.175 (1H, d, J = 7.81 Hz), 7.008 (1H, d, J = 7.81 Hz), 6.904 (1H, s), 3.420 (2H, S), 2.341 (3H, s), 2.308 (3H, s), 1.430 (9H, s) ppm.

(2-Acetoxyl-4-bromomethylphenyl)acetic acid tert-butyl ester (29). To a solution of 2-(2-acetoxyl-4-methylphenyl)acetic acid tert-butyl ester (4.9 g, 18.5 mmol) in CCl_4 (50 ml) was added N-bromosuccinimide (3.29 g, 18.5 mmol, 1 equivalents) and benzoyl peroxide (100 mg), the reaction mixture was refluxed under argon for 3 hr. The reaction mixture was then cooled to room temperature. The solid precipitate was filtered off, and washed with hexanes, the combined organic was taken to dryness, and the residue obtained was purified by chromatography (Hexanes - EtOAc, 50 : 1 to 40 : 1) to give (2-acetoxyl-4-bromomethylphenyl)acetic acid tert-butyl ester 2.467 g (38.8% yield) as an oil. ^{1}H NMR ($CDCl_3$) δ: 7.290 (1H, d, J = 7.81 Hz), 7.230 (1H, dd, J = 1.71, 7.81 Hz), 7.158 (1H, d, J = 1.71 Hz), 4.467 (2H, s), 3.462 (2H, S), 2.320 (3H, s), 1.430 (9H, s) ppm. FABMS ($^+$Ve), m/z 345 [MH^+, ^{81}Br], 343 [MH^+, ^{79}Br], 389 [$MH^+ - C_4H_8$, ^{81}Br], 387 [$MH^+ - C_4H_8$, ^{79}Br]. Anal. calcd. for $C_{15}H_{19}BrO_4$: C, 52.5; H, 5.6; Br, 23.3. Found: C, 52.30; H, 5.50; Br, 23.44.

Benzyl (3S,5S,6R)-3-{[3-acetoxyl-4-(tert-butoxycarbonylmethyl)phenyl]-methyl}-(-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate (30). To a solution of benzyl (2R,3S)-(-)-6-oxo-2,3-diphenyl-4-morpholine-carboxylate (2.47 g, 6.39 mmol) in anhydrous tetrahydrofuran (40 ml) and HMPA (4.4 ml) cooled to -78°C under argon atmosphere was added lithium bis(trimethylsilyl)amide (1.0M solution in hexanes, 6.70 ml, 6.70 mmol, 1.05 equivalents). The reaction mixture was stirred at -78°C for 1 hour. A solution of (2-acetoxyl-4-bromomethylphenyl)acetic acid tert-butyl ester (2.192 g, 6.39 mmol) in THF (10 ml) was added slowly at -78°C via a syringe, and the reaction was allowed to proceed at -78°C for 2 hours. The temperature was then raised to room temperature, and the mixture stirred overnight. The reaction mixture was quenched with aqueous NH_4Cl (10 ml) and diluted with 35 ml of water. The mixture was then extracted with ethyl acetate (50 ml x 3), and the combined organics were washed successively with water, aqueous NH_4Cl, and brine, dried over Na_2SO_4. Concentration and purification by silica gel chromatography (hexanes-ethyl acetate, from 6:1 to 3:1) gave benzyl (3S,5S,6R)-3-{[3-acetoxyl-4-(tert-butoxycarbonylmethyl)phenyl]-methyl}-(-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate as a white foam (2.070 g, 50.0% yield). ^1H NMR ($CDCl_3$) (two conformers were observed in a ratio of 2.7 : 1 at 23°C) δ: 7.396 (1H, m, overlapping), 7.279~6.959 (12H, m, overlapping), 6.869~6.696 (3H, m, overlapping; major conformer: 6.50 (2H, d, J = 7.32 Hz), 5.317 (1H, dd, J = 2.68, 6.10 Hz, -CHNCOO), 5.030 (2H, s, OCH_2Ph), 4.999 (1H, d, J = 2.93 Hz, -PhCHOOC-), 4.315 (1H, d, J = 3.18 Hz, -PhCHN-), 3.777 (1H, dd, J = 5.86, 13.67 Hz, $-CH_2$-CHNCOO), 3.547~3.30 (3H, m, overlapping, $tBuOOCCH_2$-, $-CH_2$-CHNCOO), 2.313 (3H, s), 1.411 (9H, s) ppm; minor conformer: 6.586 (2H, d, J = 7.08 Hz), 5.25 (1H, m, -CHNCOO), 5.134 (2H, s, OCH_2Ph), 5.063 (1H, d, J = 2.93 Hz, -PhCHOOC), 4.484 (1H, d, J = 2.69 Hz, PhCHN-), 3.597~3.30 (4H, m, overlapping, $tBuOOCCH_2$-, $-CH_2$-CHNCOO), 2.329 (3H, s), 1.590 (9H, s) ppm; FABMS ($^+$Ve) m/z 594 [$MH^+ - C_4H_8$], 550 [$MH^+ - C_4H_8 - CO_2$]. Anal. calcd. for $C_{42}H_{45}NO_8$: C, 72.1; H, 6.1; N, 2.2. Found: C, 72.10; H, 6.15; N, 2.13.

[3-Acetoxyl-4-(tert-butoxycarbonyl)methyl]-L-phenylalanine (31). Benzyl (3S,5S,6R)-3-{[3-acetoxyl-4-(tert-butoxycarbonylmethyl)phenyl]-methyl}-(-)-6-oxo-5,6-diphenyl-4-morpholine-carboxylate (1.50 g, 2.16 mmol) was dissolved in THF-EtOH mixture (1:2, 18 ml) and hydrogenated over Pd black (200 mg) under high pressure (50 psi ~ 20 ps or 345 kPa to about 138 kPa i) at room temperature till all the starting material was transformed to the desired product. The mixture was filtered off and the solid obtained was washed with MeOH. The combined organics was concentrated to give a white sticky solid. The solid was washed thoroughly with ether to remove 1,2-diphenylethane and dried under vacuum to 3-acetoxyl-4-(tert-butoxycarbonyl)methyl]-L-phenylalanine as a white powder (857 mg, 100%). ^{1}H NMR (DMSO) δ: 8.35 (1H, s, br), 7.274 (1H, d, J = 7.57 Hz), 7.122 (1H, d, J = 7.57 Hz), 7.049 (1H, s), 4.155 (1H, m), 3.448 (2H, s), 3.320 (2H, m), 3.093 (2H, d, J = 6.10 Hz), 2.263 (3H, s), 1.385 (9H, s) ppm; FABMS (⁻Ve) m/z 336 [M-H]. Anal. calcd. for $C_{17}H_{23}NO_6$: C, 60.5; H, 6.9; N, 4.2. Found: C, 59.09; H, 6.83; N, 3.32.

N-Fmoc-[3-acetoxyl-4-(tert-butoxycarbonyl)methyl]-L-phenylalanine (32). A mixture of [3-acetoxyl-4-(tert-butoxycarbonyl)methyl]-L-phenylalanine (857 mg, 2.16 mmol), Fmoc-OSu (727 mg, 2.16 mmol) and $NaHCO_3$ (906 mg, 10.8 mmol, 5 equivalents.) in 48 ml of dioxane-water (1:1) was stirred at room temperature overnight; the reaction mixture was then cooled to 0°C and acidified with 180 ml of 0.2 M HCl. The product was extracted with ethyl acetate (30 ml x 3), and the combined organic extract was washed with brine, dried (Na_2SO_4), and concentrated. The crude product obtained was purified by silica gel chromatography ($CDCl_3$-EtOAc-MeOH) to provide N-Fmoc-[3-acetoxyl-4-(tert-butoxycarbonyl)methyl]-L-phenylalanine as a white solid (510 mg, 39.5%).^{1}H NMR (DMSO) δ: 12.70 (1H, s, br), 7.884 (2H, d, J = 7.33 Hz), 7.773 (2H, m), 7.433~7.043 (8H, m), 4.365~4.102 (4H, m), 3.405 (2H, s), 3.200 (2H, m), 2.925~2.80 91H, m), 2.206 (3H, s), 1.356 (9H, s) ppm. FABMS (⁻Ve) m/z 711.6 [M-H=NBA], 558.5 (M-H). HR-FABMS calcd for $C_{32}H_{32}NO_6$ [M-H] m/z 558.2128.

EXAMPLE 5

This Example illustrates a method of preparing additional embodiments of formula X. The reactions involved are schematically illustrated in Fig. 16.

EXAMPLE 6

This Example illustrates the biological activity of the compounds of formula X as Grb2 SH2 domain binding inhibitors.

Cell lines were obtained from the American Type Culture Collection (Rockville, MD) and Lombardi Cancer Center, Georgetown University Medical Center. Cells were routinely maintained in improved minimal essential medium (IMEM, Biofluids, Rockville, MD) with 10% fetal bovine serum. Cultures were maintained in a humidified incubator at 37°C and 5% CO_2.

The Biacore Binding Assay

Inhibition of SH2 domain binding was determined by the Surface Plasmon Resonance (SPR) method. The solution IC_{50} values for peptide binding inhibition were measured as described in Yao et al., J. Med. Chem., 42, 25-35 (1999). Compounds **11**, **12**, and **20a** had IC_{50} average values of 155 nM, 500 nM, and 117 nM, respectively.

Assay of Cell Growth and Proliferation Inhibition by Grb2 inhibitors

The effect of Grb2 inhibitors on protein synthesis was determined by two growth assays. The first assay, inhibition of cell proliferation assays were carried out on plastics to directly measure the cell killing activity. The results obtained are set forth in Fig. 17. Cells that have amplified erbB-2 signaling such as the MDA-453 cells are inhibited by treatment with the tested inhibitors. Cells that do not utilize the activation of Grb2 or have down-stream activation of Grb2 such as MDA-231 (containing mutant ras protein) are not inhibited by treatment with the tested Grb2 inhibitors.

The second assay, a soluble tetrazolium/formazan (XTT) assay for cell growth in a 96-well plate was performed. Cells (2,000-4,000 cells/well) were grown in IMEM medium with 10% FBS and were treated with increasing concentrations of Grb2 inhibitors(1-50 uM). After 6-8 days culture, XTT (1.0 mg/ml plus PMS at 1.53 mg/ml) was added to each well and incubated for four hours at 37°C. Absorbance at 450 nm was measured with the Dynatech

Model MR700. The results obtained showed that the compounds of the present invention have cell killing activity.

Inhibition of MAP Kinase.

MAP kinases function in a protein kinase cascade that plays a critical role in the regulation of cell growth and differentiation. MAP kinases are activated by a wide variety of signals including growth factors, cytokines and hormones through Grb2 and other signaling proteins. The inhibition of MAP kinase in MDA-453 cells treated with growth factor heregulin (HRG) by MAP kinase specific antibody was measured. 1-2 $x10^6$ cells were plated into 100 mm dishes with 10% FBS. Cells were washed twice with ice-cold PBS and lysed in 1 ml of lysis buffer (50 mM Tris-HCL, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100, 5 mM EDTA, 5 mM EGTA, 1 mM PMSF, 50 μg/ml approtinin, 50 μg/ml leupeptin, and 2 mM sodium orthovanadate). The protein concentration was determined by BCA method (Pierce, Rockford, IL). 50 μg of protein was subjected to 8-20% SDS-PAGE gel (Novex, San Diego, CA) and transferred to a nitrocellulose membrane. Activation of MAP kinase was detected with a specific antibody, i.e., phospho-p44/42 MAP kinase antibody (New England BioLabs) and visualized with ECL (Amersham, Arlington Heights, IL). The blotting results obtained confirmed that over 60% inhibition was achieved with compound **11**.

The Grb2 binding inhibition also was determined by carrying out an ELISA assay. The results obtained are set forth in Fig. 19. Compounds **11** and **12** were effective in inhibiting Grb2 binding.

In a separate set of experiments, the Biacore Binding Assay was compared to an ELISA assay, and excellent agreement was observed between the two assays.

EXAMPLE 7

This Example illustrates an advantage of the compounds of the present invention. When used in conjunction with chemotherapeutic drugs, synergistic effects have been observed.

Compound 126 inhibited colony formation of HBC BT-474 and MDA-453 cell lines. The soft agar colony formation was tested as follows. Cells in

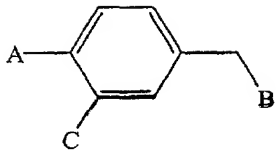
suspension (10,000 cells/ml) were mixed with 0.33% agarose and plated on top of a layer with 1% agarose. The next day, different concentrations of the inhibitor mixed with 1 ml of the culture medium were added to the top layer and incubated for two weeks. The number of colonies greater than 80 μm formed were counted on a Bausch and Lomb Image analysis system. In the combination therapy, chemotherapeutic drugs were mixed with 1 ml of the culture medium and were added to the top layer and incubated for two weeks.

Fig. 20 depicts the synergistic effect observed when compound 126 was used in conjunction with the chemotherapeutic drugs on the HBC BT-474 cell line. Treatment with paclitaxel ("TXT" in the Figure), doxorubicin ("DOX"), and 5-fluorouracil ("5-Fu") in combination with the inhibitor resulted in a greater inhibition of Her-2/neu-overexpressing cancer cells than that was observed with the chemotherapeutic drug alone.

The references cited herein are hereby incorporated by reference in their entireties. While this invention has been described with an emphasis upon several embodiments, it will be obvious to those of ordinary skill in the art that variations of the embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

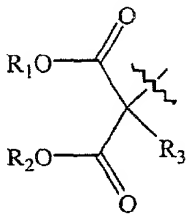
1. A compound of formula I:



(I),

wherein:

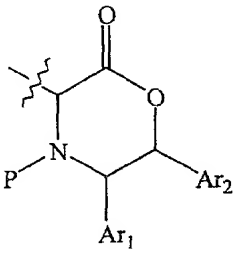
A is carboxyl, carboxyalkyl, dicarboxyalkyl, alkoxycarbonyl, alkoxycarbonylalkyl, dialkoxycarbonylalkyl, or a malonyl group of formula II:



(II),

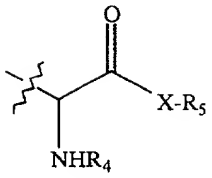
wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, alkyl, aryl, and alkoxy;

B has the formula III:



(III),

wherein P is an amine protective group; and Ar_1 and Ar_2 are aryl groups; or the formula IV:



(IV),

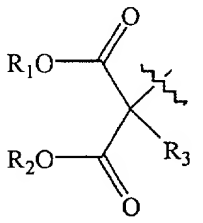
wherein X is NH or O; R_4 is hydrogen, alkyl, aryl, alkylaryl, arylalkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, alkyl, aryl, arylalkyl, alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, alkyl, alkylcarbonyl, alkylcarbonyloxy, alkoxycarbonyl, and alkoxycarbonyl alkyl;

wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of alkyl, hydroxy, halo, keto, amino, and alkoxy; with the provisos that (i) R_5 is not hydrogen when A is carboxyl or carboxyalkyl, C is hydrogen, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is NH; and (ii) R_5 is not hydrogen or alkyl when A is carboxyl or carboxyalkyl, C is hydrogen or hydroxy, B has the formula IV wherein R_4 is hydrogen or alkylcarbonyl, and X is O.

2. The compound of claim 1, wherein:

A is carboxyl, carboxyl C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyl, C_1-C_6 alkoxycarbonyl, C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl, C_1-C_6 dialkoxycarbonyl C_1-C_6 alkyl, or a malonyl group of formula II:

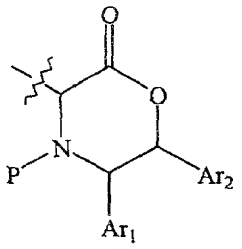


(II),

wherein R_1 and R_2 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6

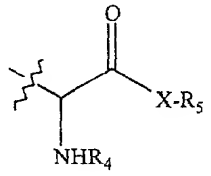
alkylaryl, and heteroaryl; and R_3 is selected from the group consisting of hydrogen, halo, hydroxy, amino, C_1-C_6 alkyl, aryl, and C_1-C_6 alkoxy;

B has the formula III:



(III),

wherein P is an amine protective group; and Ar_1 and Ar_2 are aryl groups; or B has the formula IV:



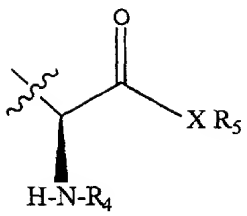
(IV),

wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl; and

C is selected from the group consisting of hydrogen, hydroxyl, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, C_1-C_6 alkylcarbonyloxy, C_1-C_6 alkoxycarbonyl, and C_1-C_6 alkoxycarbonyl C_1-C_6 alkyl; wherein said aryl, heteroaryl, and the aryl portion of said arylalkyl and alkylaryl may be unsubstituted or substituted with a substituent selected from the group consisting of C_1-C_6 alkyl, hydroxy, halo, keto, amino, and C_1-C_6 alkoxy.

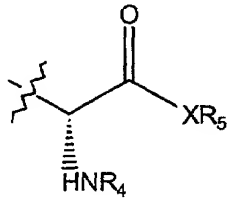
3. The compound of claim 2, wherein B has the formula IV.

4. The compound of claim 3, wherein B has the formula:



wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

5. The compound of claim 3, wherein B has the formula:



wherein X is NH or O; R_4 is hydrogen, C_1-C_6 alkyl, aryl, C_1-C_6 alkylaryl, aryl C_1-C_6 alkyl, or an amine protective group; and R_5 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkylaryl, and heteroaryl.

6. The compound of claim 4 or 5, wherein X is O.

7. The compound of claim 6, wherein R_4 is hydrogen.

8. The compound of claim 6, wherein R_4 is an amine protecting group.

9. The compound of claim 8, wherein acid amine protecting group is selected from the group consisting of fluorenylmethoxycarbonyl, tert-butoxycarbonyl, carbobenzoxy, and carbamoyl.

10. The compound of claim 8, wherein R_5 is hydrogen.

11. The compound of any of claims 4-10, wherein R_1 and R_2 are hydrogen.

12. The compound of claim 11, wherein R_3 is hydrogen.

13. The compound of any of claims 4-12, wherein C is hydrogen.

14. The compound of any of claims 4-12, wherein C is C_1-C_6 alkylcarbonyl.

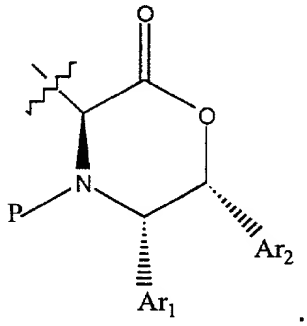
15. The compound of any of claim 4-12 and 14, wherein C is tert-butoxycarbonyl.

16. The compound of any of claims 4-12, wherein C is C_1-C_6 alkylcarbonyloxy.

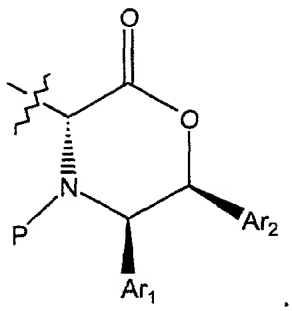
17. The compound of any of claims 4-12 and 16, wherein C is acetyloxy.

18. The compound of claim 1 or 2, wherein B has the formula III.

19. The compound of claim 18, wherein B has the formula:



20. The compound of claim 18, wherein B has the formula:

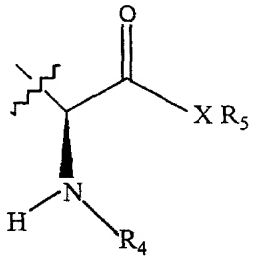


21. The compound of claim 19 or 20, wherein Ar_1 and Ar_2 are phenyl.

22. The compound of any of claims 18-21, wherein said amine protecting group is selected from the group consisting of fluorenylmethoxycarbonyl, tert-butoxy carbonyl, carbobenzoxy, and carbamoyl.

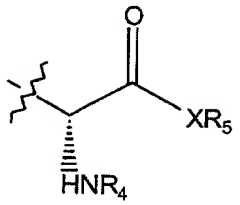
23. The compound of claim 9 or 22, wherein said amine protecting group is fluorenylmethoxycarbonyl.

24. The compound of claim 1 or 2, wherein R_1 and R_2 are tert-butyl and R_3 is hydrogen, and B has the formula



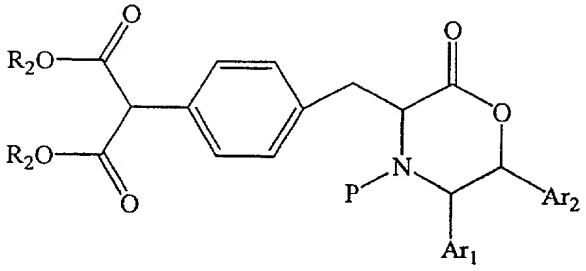
wherein X is O, R_4 is fluorenylmethoxycarbonyl, and R_5 is hydrogen.

25. The compound of claim 1 or 2, wherein R_1 and R_2 are tert-butyl, and R_3 is hydrogen; and B has the formula



wherein R_4 is fluorenylmethoxycarbonyl.

25. A process for the preparation of a compound of formula VII:



(VII),

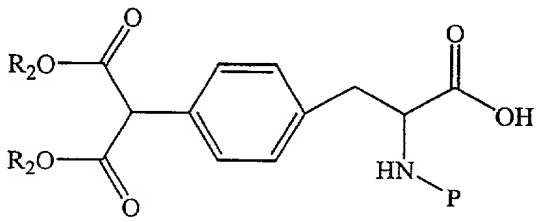
wherein R_2 is alkyl, P is an amine protecting group, and Ar_1 and Ar_2 are aryl; the process comprising:

(a) converting a p-halotoluene to a p-tolyl-malonic acid dialkyl ester by contacting the p-halotoluene with a dialkylmalonate and a cuprous halide;

(b) halogenating the p-tolyl-malonic acid dialkyl ester to obtain a (4-halomethylphenyl)-malonic acid dialkyl ester; and

(c) contacting the (4-halomethylphenyl)-malonic acid dialkyl ester with a benzyl-6-oxo-2,3-diaryl-4-morpholine to obtain the compound of formula VII.

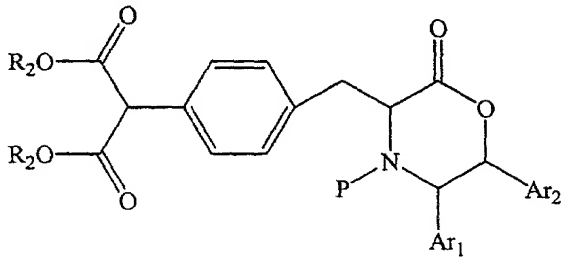
26. A process for preparing a compound of formula VIII:



(VIII),

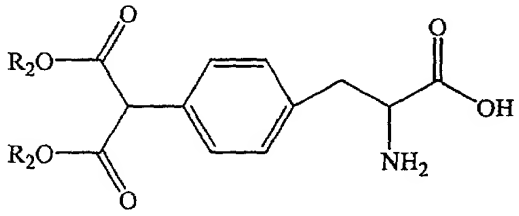
wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing the compound of formula



(VII),

to obtain a compound of formula IX:

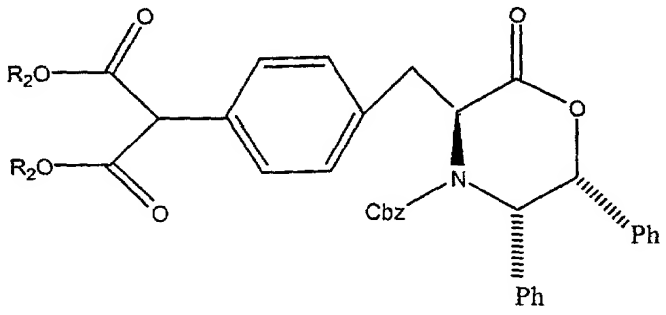


(IX);

and

(b) reacting the compound of formula IX with an amine protecting agent to obtain the compound of formula VIII.

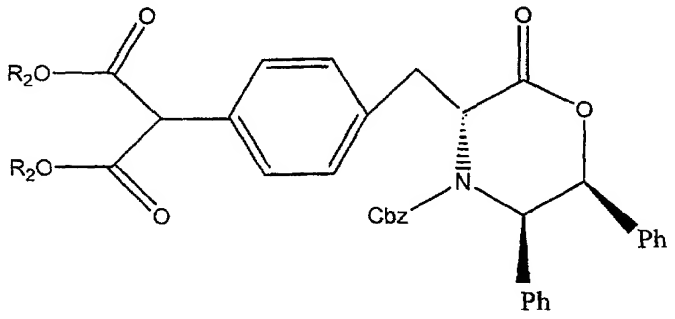
27. The process of claim 26, wherein the compound of formula VII is:



(VIIa)

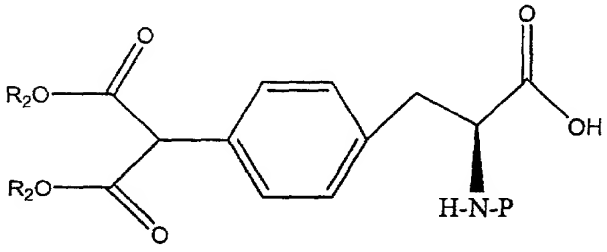
wherein said benzyl-6-oxo-2,3-diphenyl-4-morpholine is benzyl (2R,3S)- (-) - 6-oxo-2,3-diphenyl-4-morpholine.

28. The process of claim 26, wherein the compound of formula VII is:



(VIIb)

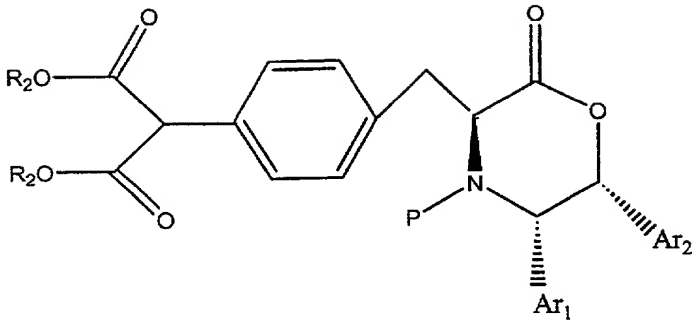
29. A process for preparing a compound of formula VIIIa:



(VIIIa)

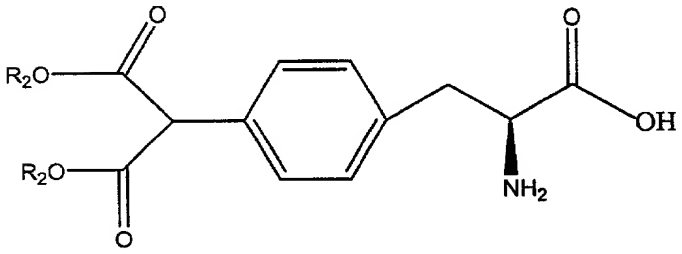
wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing a compound of formula VII



(VIIa)

to obtain a compound of formula IXa:

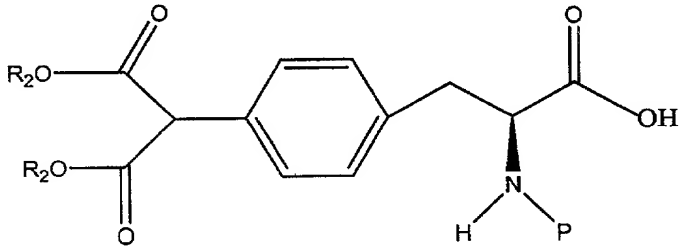


(IXa);

and

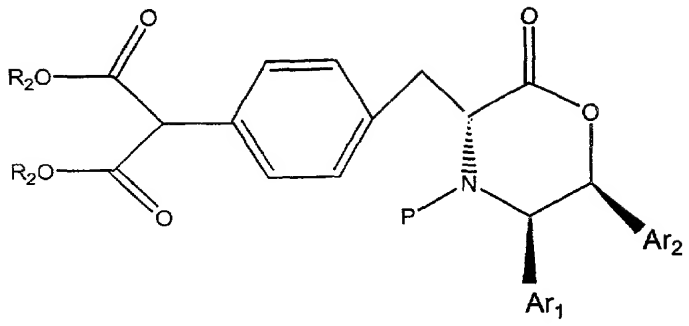
(b) reacting the compound of formula IXa with an amine protecting agent to obtain the compound of formula VIII.

30. A process for preparing a compound of the formula:

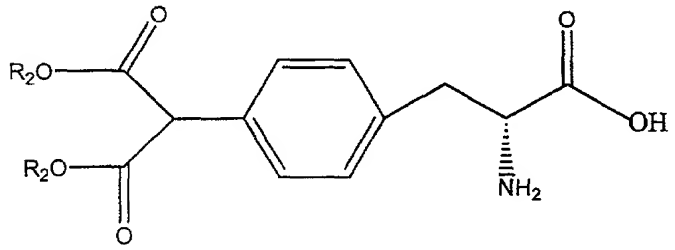


wherein R_2 is alkyl and P is an amine protecting group; the process comprising:

(a) reducing a compound of formula:



to obtain a compound of formula IXb:



(IXb);

and (b) reacting the compound of formula IXa with an amine protecting agent to obtain the compound of formula VIII.

31. The process of claim 27, wherein said p-halotoluene is p-iodotoluene.

32. The process of claim 27, wherein said (4-halomethylphenyl)-malonic acid dialkyl is (4-bromomethylphenyl)-malonic acid dialkyl ester.

33. The process of any of claims 27-32, wherein R_2 is t-butyl.

34. A conjugate comprising a conjugant covalently linked to a compound of any of claims 1-25.

35. The conjugate of claim 34, wherein said conjugant is an amino acid or a polypeptide.

36. The conjugate of claim 34, wherein said conjugant is a nucleic acid or a nucleotide.

37. The conjugate of claim 34, wherein said conjugant is a polymer.

38. A compound of the formula:

$$W\text{-}Y\text{-}(AA)_n\text{-}Z$$

wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxyalkyl, carboxyalkyloxy, dicarboxyalkyl, dicarboxyalkyloxy, dicarboxyhaloalkyl, dicarboxyhaloalkyloxy, and phosphonoalkyl, phosphonohaloalkyl, wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto;

W is a moiety attached to the nitrogen of Y and is selected from the group consisting of alkylcarbonyl, oxalyl, alkylaminooxalyl, arylaminooxalyl, arylalkylaminooxalyl, alkoxyoxalyl, carboxyalkyl carbonyl, heterocyclyl carbonyl, heterocyclylalkyl carbonyl, arylalkyl heterocyclylalkyl carbonyl, aryloxycarbonyl, and arylalkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, alkyl, alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an arylalkylamino or arylheterocyclyl alkylamino;

or a salt thereof;

with the proviso that W is not arylalkylamino when the phenyl ring of phenylalanyl contains a phosphonoalkyl or phosphonohaloalkyl substituent at a position para to the alkylamido group and the ortho and meta positions are unsubstituted.

39. The compound of claim 38, wherein n is 0 to 15;

Y is a phenylalanyl radical having a phenyl ring, an amine end, and a carboxyl end, the phenyl ring having one or more substituents selected from the group consisting of hydroxyl, carboxyl, formyl, carboxy C_1-C_6 alkyl, carboxy C_1-C_6 alkyloxy, dicarboxy C_1-C_6 alkyl, dicarboxy C_1-C_6 alkyloxy, dicarboxyhalo C_1-C_6 alkyl, dicarboxyhalo C_1-C_6 alkyloxy, and phosphono C_1-C_6 alkyl, phosphonohalo C_1-C_6 alkyl, wherein the alkyl portion of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, aminoalkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto;

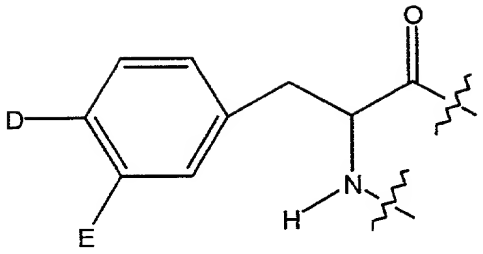
W is a moiety attached to the nitrogen of Y and is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S;

AA is an amino acid, the amine end of which is attached to the carboxyl end of Y; and

Z is an aryl C_1-C_6 alkylamino or arylheterocyclyl C_1-C_6 alkylamino;

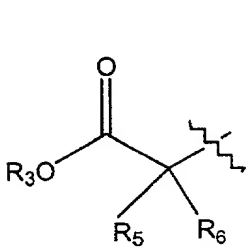
or a salt thereof.

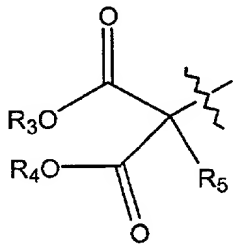
40. The compound of claim 39, wherein Y is of the formula XI:

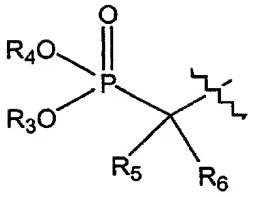


(XI)

wherein D has the formula XII, XIII, or XIV:







(XII) (XIII) (XIV)

wherein R_3 and R_4 may be the same or different and are selected from the group consisting of hydrogen, C_1-C_6 alkyl, aryl, aryl C_1-C_6 alkyl, C_1-C_6 alkaryl, and heteroaryl; and R_5 and R_6 may be the same or different and are selected from the group consisting of hydrogen, halo, hydroxy, amino, and C_1-C_6 alkoxy; and

E is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, carboxyl, and C_1-C_6 alkylcarbonyl C_1-C_6 alkyl.

41. The compound of claim 40, wherein D is of formula XII.

42. The compound of claim 40, wherein D is of formula XIII.

43. The compound of claim 40, wherein D is of formula XIV.

44. The compound of any of claims 41-43, wherein E is hydrogen.

45. The compound of claim 41, wherein E is carboxyl.

46. The compound of any of claim 41-45, wherein R_3, R_4, R_5, and R_6 are hydrogen.

47. The compound of claim 43, wherein R_3 and R_4 are hydrogen.

48. The compound of any of claims 38-47, wherein W is selected from the group consisting of C_1-C_6 alkylcarbonyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxy C_1-C_6 alkyl carbonyl, heterocyclyl carbonyl, heterocyclyl C_1-C_6 alkyl carbonyl, aryl C_1-C_6 alkyl heterocyclyl C_1-C_6 alkyl carbonyl, aryloxycarbonyl, and aryl C_1-C_6 alkoxycarbonyl, wherein the aryl and alkyl portions of the substituents may be unsubstituted or substituted with a substituent selected from the group consisting of halo, hydroxy, carboxyl, amino, amino C_1-C_6 alkyl, C_1-C_6 alkyl, C_1-C_6 alkoxy, and keto; and the heterocyclyl portion of W contains at least 4 hetero atoms selected from the group consisting of O, N, and S.

49. The compound of any of claims 38-48, wherein W is C_1-C_6 alkyloxycarbonyl.

50. The compound of any of claims 38-49, wherein W is acetyl.

51. The compound of any of claims 38-48, wherein W is oxalyl.

52. The compound of any of claims 38-48, wherein W is carboxymethylcarbonyl.

53. The compound of any of claims 38-48, wherein W is tetrazolylcarbonyl.

54. The compound of any of claims 38-48, wherein W is tetrazolylmethylcarbonyl.

55. The compound of any of claims 38-48, wherein W is an arylmethyloxycarbonyl.

56. The compound of any of claims 38-48 and 55, wherein W is an aminophenylmethyloxycarbonyl.

57. The compound of any of claims 38-48 and 55-56, wherein W is 3-aminophenyl-1-methyloxycarbonyl.

58. The compound of any of claims 38-48, wherein W is an aryloxycarbonyl.

59. The compound of any of claims 38-48 and 58, wherein W is an naphthyloxycarbonyl.

60. The compound of any of claims 38-48 and 58-59, wherein W is an aminonaphthyloxycarbonyl.

61. The compound of any of claims 38-48 and 58-60, wherein W is 6-amino-1-naphthyloxycarbonyl.

62. The compound of any of claims 38-48, wherein W is an arylmethyltetrazolylmethylcarbonyl.

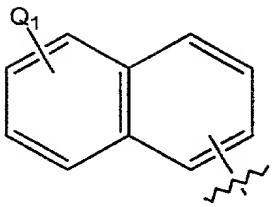
63. The compound of any of claims 38-48 and 62, wherein W is a phenylmethyltetrazolylmethylcarbonyl.

64. The compound of any of claims 38-48 and 62-63, wherein W is an alkoxyphenylmethyltetrazolylmethylcarbonyl.

65. The compound of any of claims 38-48 and 62-64, wherein W is a methoxyphenylmethyltetrazolylmethylcarbonyl.

66. The compound of any of claims 38-65, wherein Z is aryl C_1-C_6 alkylamino.

67. The compound of claim 66, wherein the aryl portion of Z has the formula:



wherein Q_1 is hydrogen or a substituent selected from the group consisting of hydroxyl, halo, C_1-C_6 alkyl, C_1-C_6 alkoxy, amino, and C_1-C_6 acylamino.

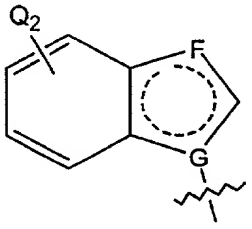
68. The compound of claim 67, wherein the aryl portion of Z is attached to the alkylamino portion of Z at the aryl 1- or 2- position.

69. The compound of claim 67 or 68, wherein Q_1 is methyl.

70. The compound of any of claims 67-69, wherein Z is naphthylpropylamino.

71. The compound of any of claims 38-65, wherein Z is aryl heterocyclyl C_1-C_6 alkylamino.

72. The compound of claim 71, wherein the heterocyclyl portion of Z has the formula:



wherein Q_2 is hydrogen or a substituent selected from the group consisting of hydroxyl, halo, C_1-C_6 alkyl, C_1-C_6 alkoxy, amino, and C_1-C_6 acylamino, and F and G are independently selected from the group consisting of C, N, O, and S.

73. The compound of claim 72, wherein F is C and G is N.

74. The compound of claim 72 or 73, wherein Q_2 is methyl.

75. The compound of any of claims 38-39, wherein W is selected from the group consisting of acetyl, oxalyl, C_1-C_6 alkylaminooxalyl, arylaminooxalyl, aryl C_1-C_6 alkylaminooxalyl, C_1-C_6 alkoxyoxalyl, carboxymethylcarbonyl, tetrazolylcarbonyl, tetrazolylmethylcarbonyl, aminophenylmethoxycarbonyl, amino naphthyloxycarbonyl, and methoxyphenylmethyl tetrazolylmethylcarbonyl.

76. The compound of any of claims 38-75, wherein n is 1-3.

77. The compound of any of claims 38-76, wherein said amino acid is selected from the group consisting of glycine, alanine, valine, norvaline, leucine, iso-leucine, norleucine, α-amino n-decanoic acid, serine, homoserine, threonine, methionine, cysteine, S-acetylaminomethyl-cysteine, proline, trans-3- and trans-4-hydroxyproline, phenylalanine, tyrosine, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, tryptophan, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aspartic acid, asparagine, aminomalonic acid, aminomalonic acid monoamide, glutamic acid, glutamine, histidine, arginine, lysine, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid and α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

78. The compound of claim 77, wherein said amino acid is selected from the group consisting of glycine, alanine, leucine, iso-leucine, norleucine, cyclohexylalanine, 4-aminocyclohexylglycine, 4-acetylaminocyclohexylglycine, aspartic acid, asparagine, glutamic acid, and glutamine.

79. The compound of any of claims 76-78, wherein n is 2.

80. The compound of claim 79, comprising a first amino acid (AA_1) attached to the phenyl alanine moiety and asparagine attached to said AA_1, wherein said AA_1 is selected from the group consisting of cyclohexylglycine, 4-aminocyclohexylglycine, and 4-acetylaminocyclohexylglycine.

81. The compound of claim 80, wherein said AA_1 is cyclohexylglycine.

82. The compound of claim 81, wherein D is of formula XIII, E, R_3, R_4, and R_5 are hydrogen, R_1 is oxalyl, and Z is naphthylpropylamino.

83.The compound of claim 38 or 39, wherein Z is not indolylpropylamino when W is acetyl, and Y is a phenylalanyl radical having a phosphonomethyl substituent.

84. A composition comprising a pharmacologically acceptable carrier and a compound of any of claims 38-83.

85. A method for inhibiting an SH2 domain from binding with a phosphoprotein comprising contacting an SH2 domain with a compound of any of claims 34-83.

86. The method of claim 85, wherein said SH2 domain is in a mammal, and said compound is administered to said mammal.

87. The use of a compound of any of claims 34-83 in the manufacture of a medicament for the treatment of a condition that responds to the inhibition of phosphoprotein binding to an SH2 domain of a mammal.

88. The use of a compound of any of claims 34-83 in medicine.

89. A compound of any of claims 34-83 for use as a Grb2-SH2 domain inhibitor.

90. A method for inhibiting SH2 domain binding comprising exposing a material containing an SH2 domain to a compound of any of claims 34-83.

91. A method for determining the presence of an SH2 domain in a material comprising:
(a) exposing a sample of said material to a SH2 binding compound and obtaining a first binding result;
(b) exposing another sample of said material to a compound of any of claims 34-83 and obtaining a second binding result; and
(c) comparing the first and second binding results to determine whether an SH2 domain is present in the material.

92. A method of preventing or treating a disease, state, or condition in a mammal comprising administering a compound of any of claims 34-83.

93. The method of claim 92, wherein the disease, state, or condition involves an SH2 domain binding.

94. The method of any of claims 86, 92 or 93, wherein the mammal is afflicted with a cancer.

95. The method of claim 94, wherein the cancer is a breast cancer or ovarian cancer.

96. The method of claim 86, 92, or 93, wherein the mammal is afflicted with a tumor.

97. The method of claim 96, wherein the tumor is leukemia or lymphoma.

98. The method of claim 96, wherein the tumor is a solid tumor.

99. The method of claim 98, wherein the solid tumor is a brain tumor or a prostate tumor.

100. The method of claim 86, 92 or 93, wherein the mammal is afflicted with an autoimmune disease.

101. The method of claim 86, 92, or 93, wherein the mammal is afflicted with an inflammatory disease.

102. The method of claim 86, 92, or 93, wherein the mammal is afflicted with diabetes.

103. The method of claim 86, 92, or 93, wherein the mammal is afflicted with obesity.

104. The method of claim 86, 92, 93, wherein the mammal is afflicted with a metabolic disease.

105. The method of claim 86, 92, or 93, wherein the mammal is afflicted with a cardiovascular disease.

106. A method of enhancing the therapeutic effect of a treatment rendered to a mammal that has been afflicted with a disease, state, or condition, comprising administering to the mammal a compound of any of claims 34-83 in conjunction with the treatment.

107. The method of claim 106, wherein the treatment comprises chemotherapy, radiation therapy, or biological therapy.

108. The method of claim 107, wherein the biological therapy comprises the use of a protein.

109. The method of claim 106 or 107, wherein the biological therapy comprises the use of an antibody or a recombinant protein.

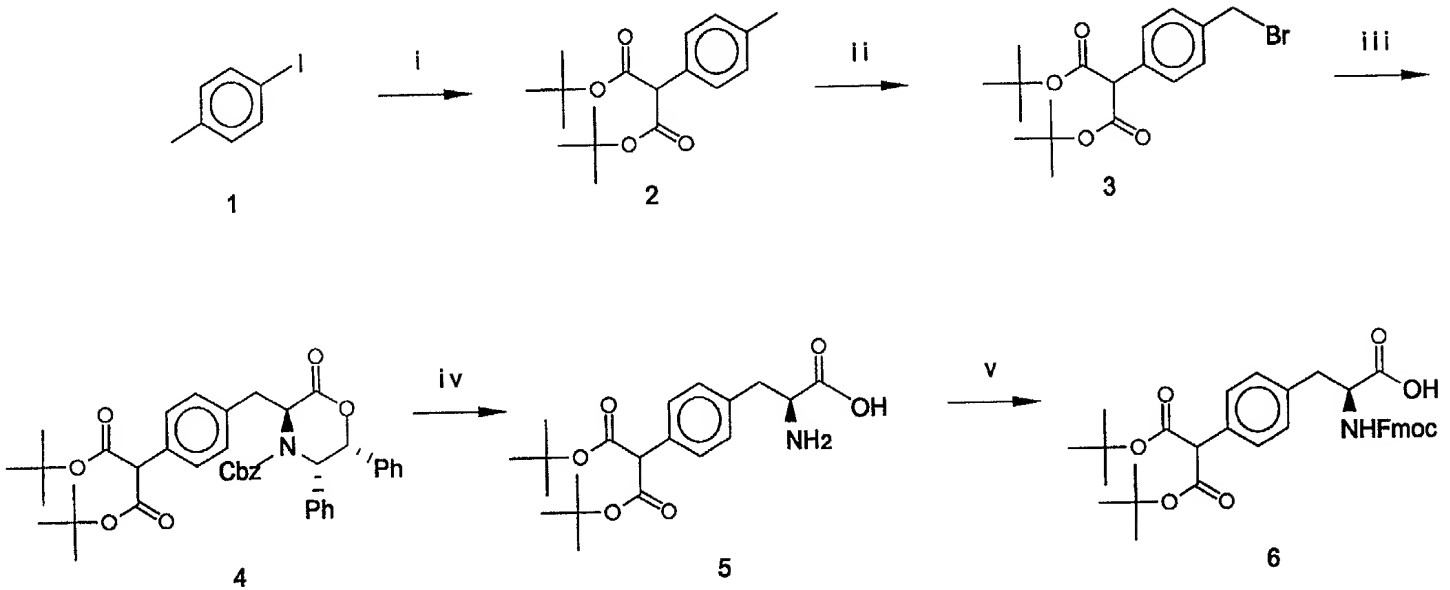
110. The method of any of claims 106-109, which comprises inhibiting a cell survival factor in the mammal.

111. The method of any of claims 106-109, which comprises triggering cell apoptosis.

112. A method of inhibiting the MAP kinase activity in a mammal comprising administering to the mammal a compound of any of claims 34-83.

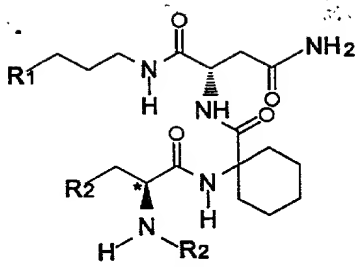
113. The method of claim 94, wherein the cancer is mediated through BCR-Abl.

114. The method of claim 92 or 93, which involves inhibiting the expression of erbB-2 receptor.



i. Di-tert-butyl malonate, NaH, CuBr, Dioxane, HMPA, 101°C, 55.2%: ii. NBS, 83.2%;
iii, Benzyl (2R, 3S)-(-)-6-oxo-2, 3-diphenyl-4-morpholine, LiHMDS, THF, -78°C-R.T., 39.2%;
iv, H_2,Pd black,86.3%; v. Fmoc-OSu, Dioxane-H_2O , $NaHCO_3$, 30-35°C, 50.7%.

FIG. 1



General Structure
(*D, L and racemate claimed)

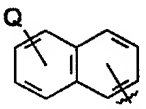
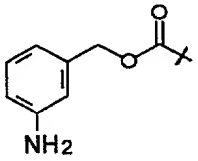
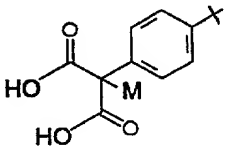
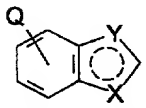
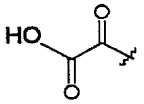
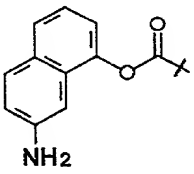
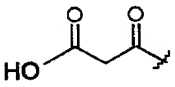
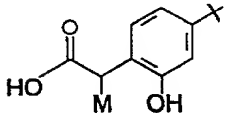
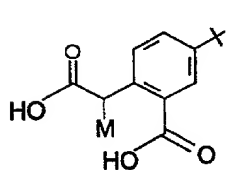
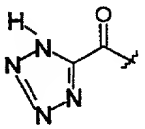
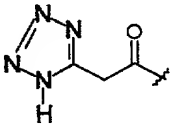
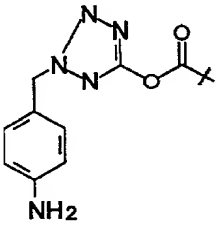
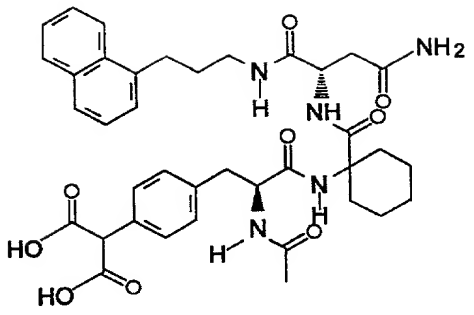
R_1	R_2	R_3
 1 and 2-substitued naphthyl	 	
 where Q=Me, short alkyl, halogen, hydroxy, alkyloxy, amino, acylamino	  	  where M= hydroxy, alkyloxy, halogen, keto, short alkyl
	  	

FIG. 2



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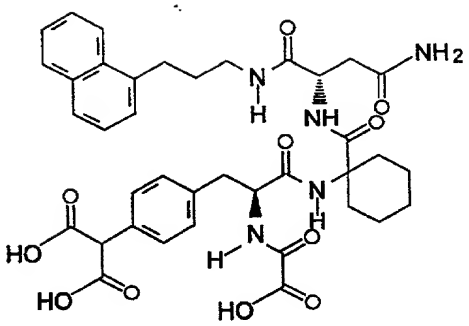

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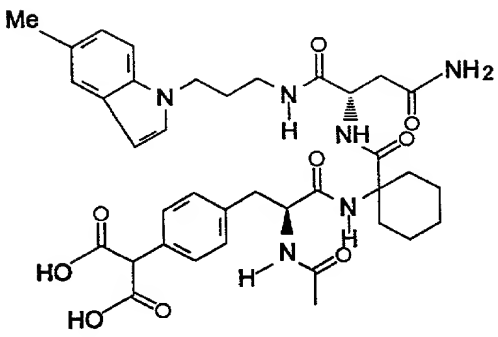

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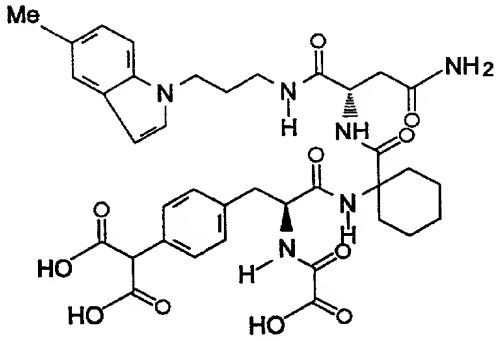

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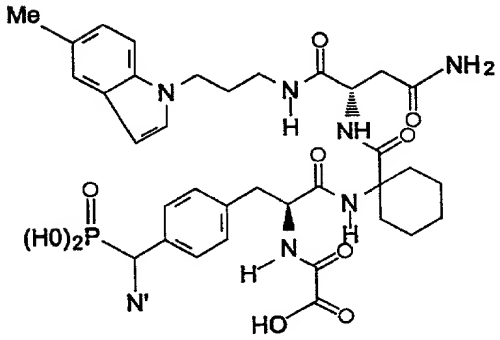
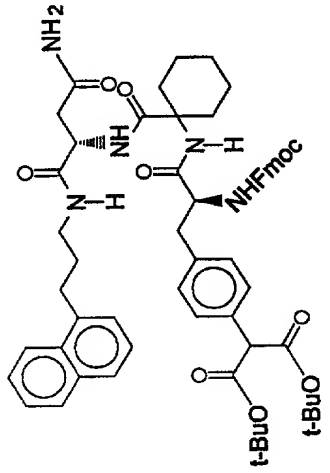


N'=H, halogen, hydroxy, F_2

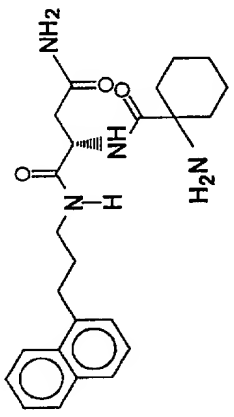
20(a, b, c,d)

FIG. 3



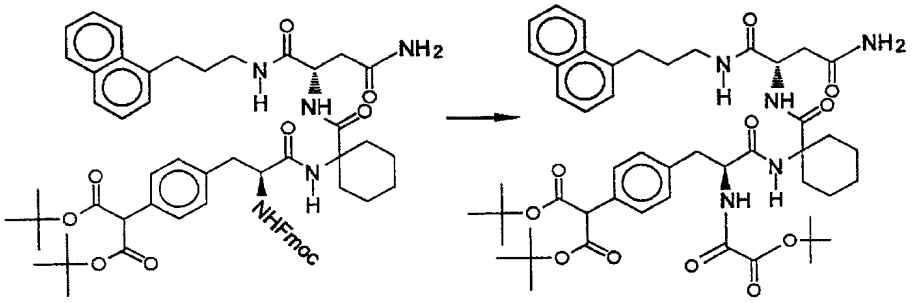
8

106C-56-2, HOBt
DIPCDI, DMF, 100%



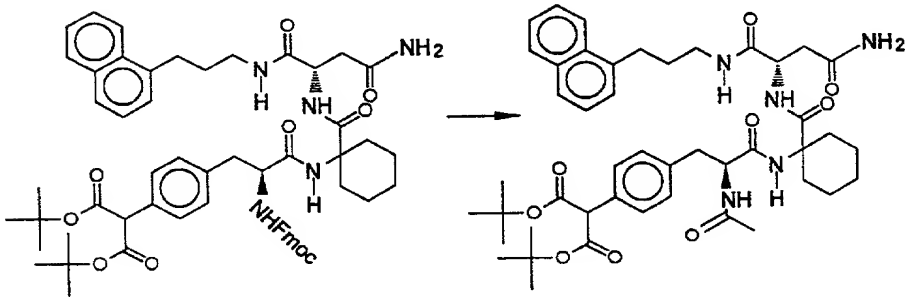
7

FIG. 4



8 9

FIG. 5



8 10

FIG. 6

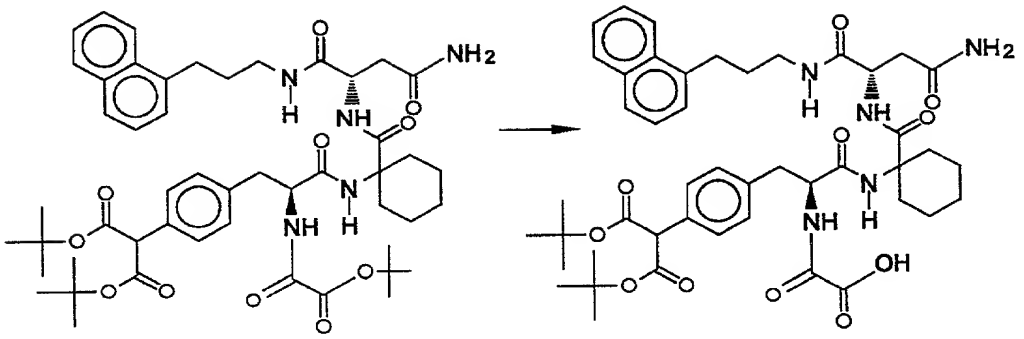


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FIG. 7

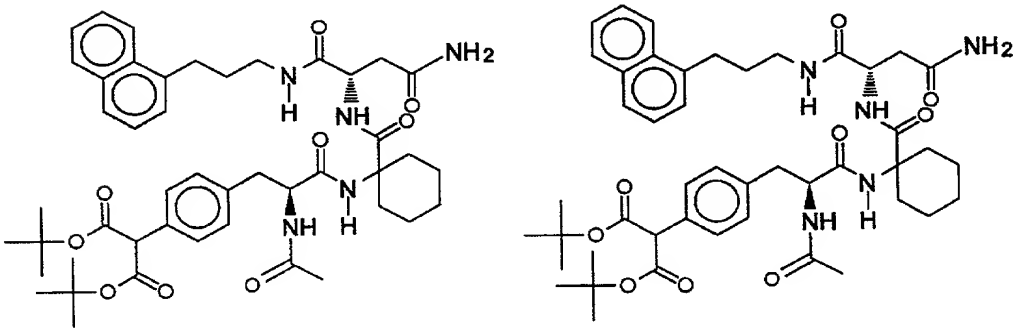

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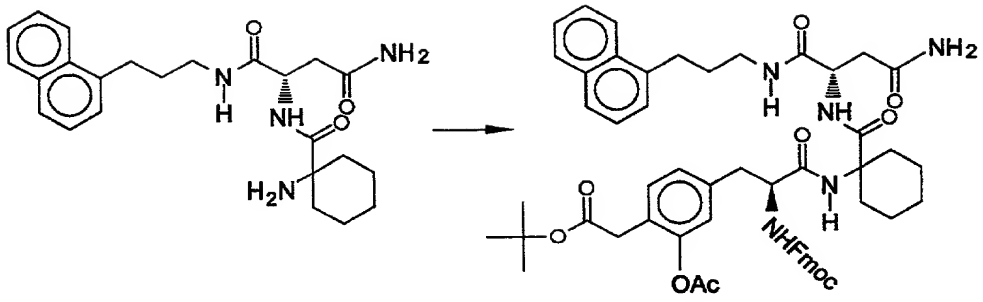
FIG. 8



7 13

FIG. 9

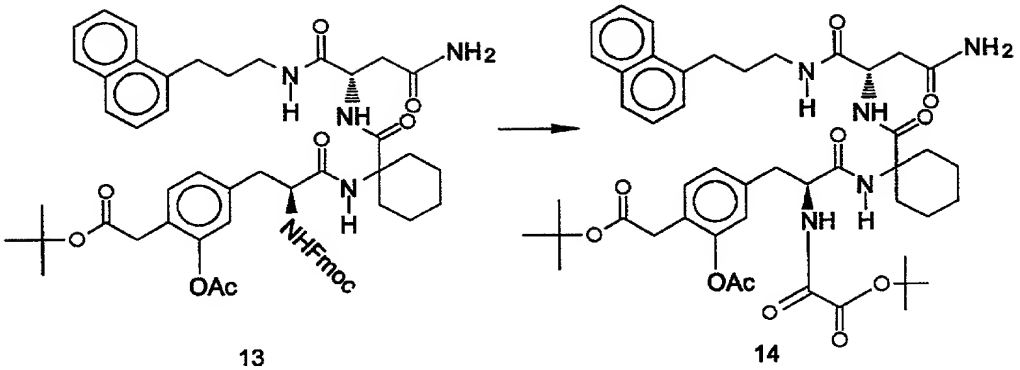


FIG. 10

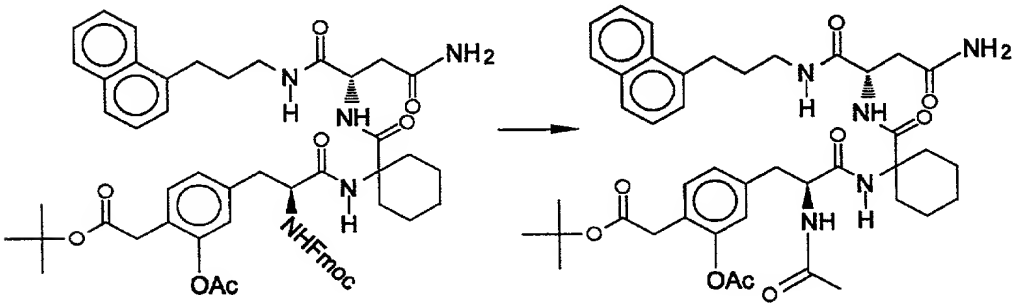
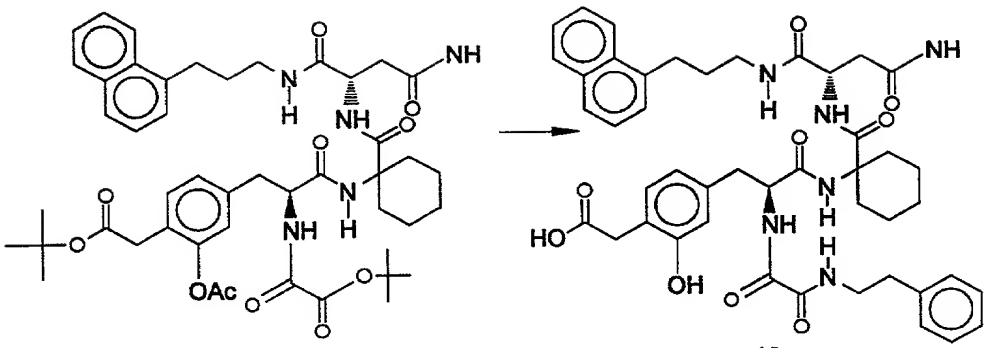
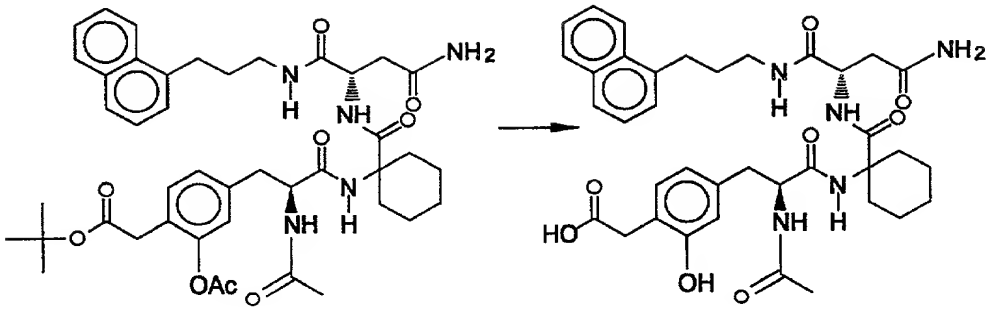


FIG. 11



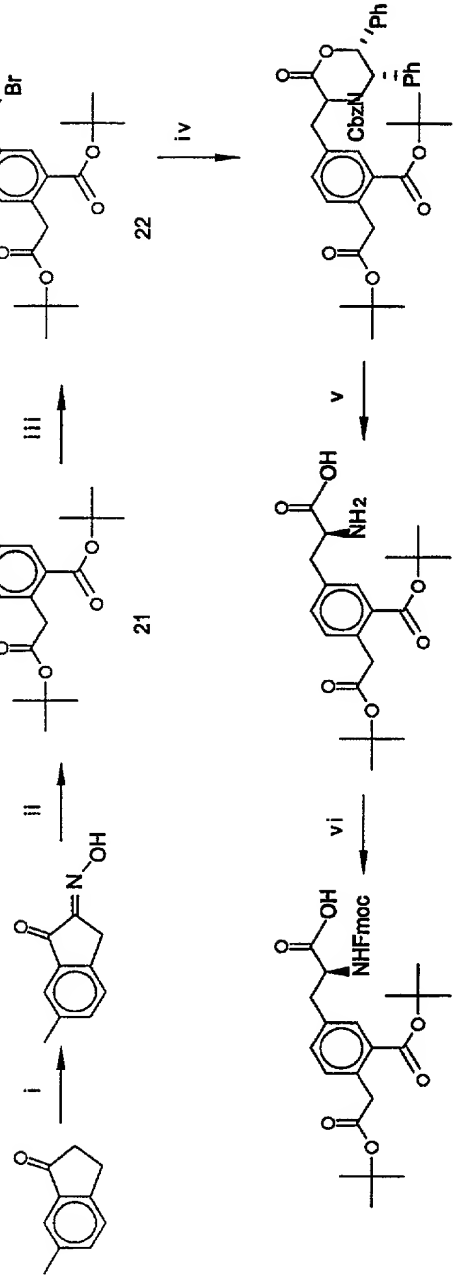
14 16

FIG. 12



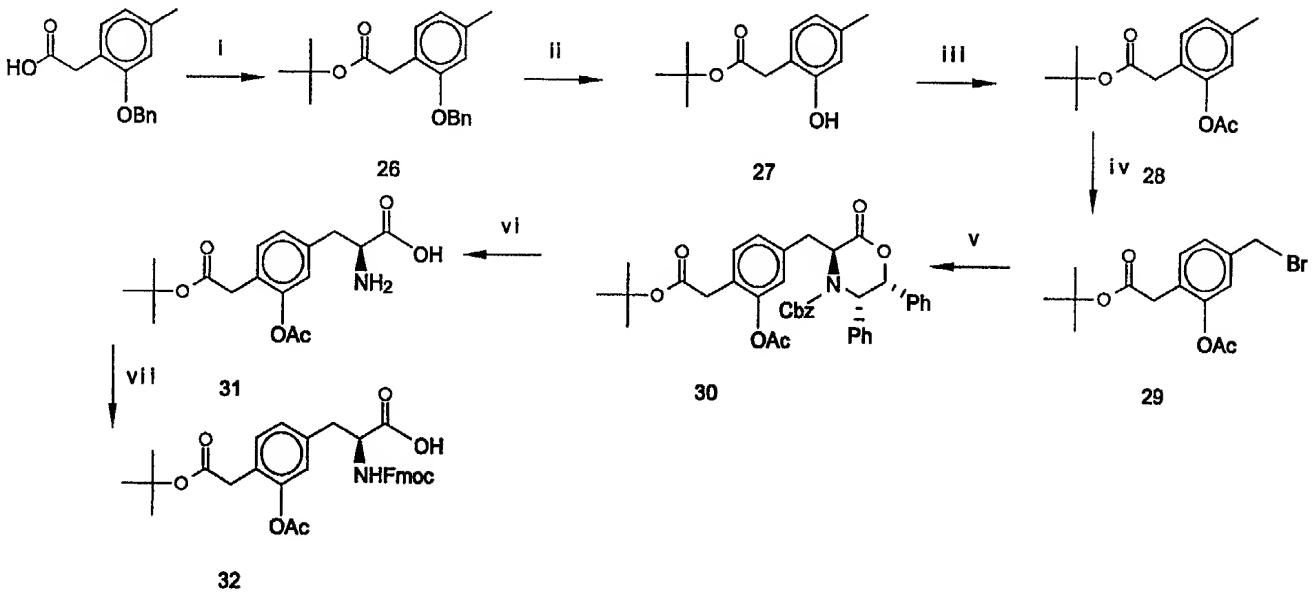
15 17

FIG. 13



i). isopentyl nitrite, concd. HCl, 82.5%; ii). a. PCl3, anhyd. ether, b. NaOH-H_2O, 89.4%
iii). 2,2,2-trichloroacetimide (t-butyl). BBr_3, CH_2Cl_2-cyclohexane, 83.3%;
iv. William's reagent, LiHMDS, HMPA, THF, 68.7%; v. H_2, Pd Black, EtOH-THF, 56.6%
vi. Fmoc-OSu, $NaHCO_3$, Dioxane-H_2O, 78.4%

FIG. 14



i). 1. $(COCl)_2$, 2. t-Butanol, 92.8%; ii). H_2, Pd black, EtOH, 100%; iii). Ac_2O, Pyr., 94%;
iv). NBS $(BzO)_2$, 38.8%; v). William's reagent. LiHMDS, THF, -78°C-R.T., 50.5%;
vi) H_2, Pd Black, EtOH-THF, 100%; vii). Fmoc-Osu, $NaHCO_3$, Dioxane-H_2O, 39.5%

FIG. 15

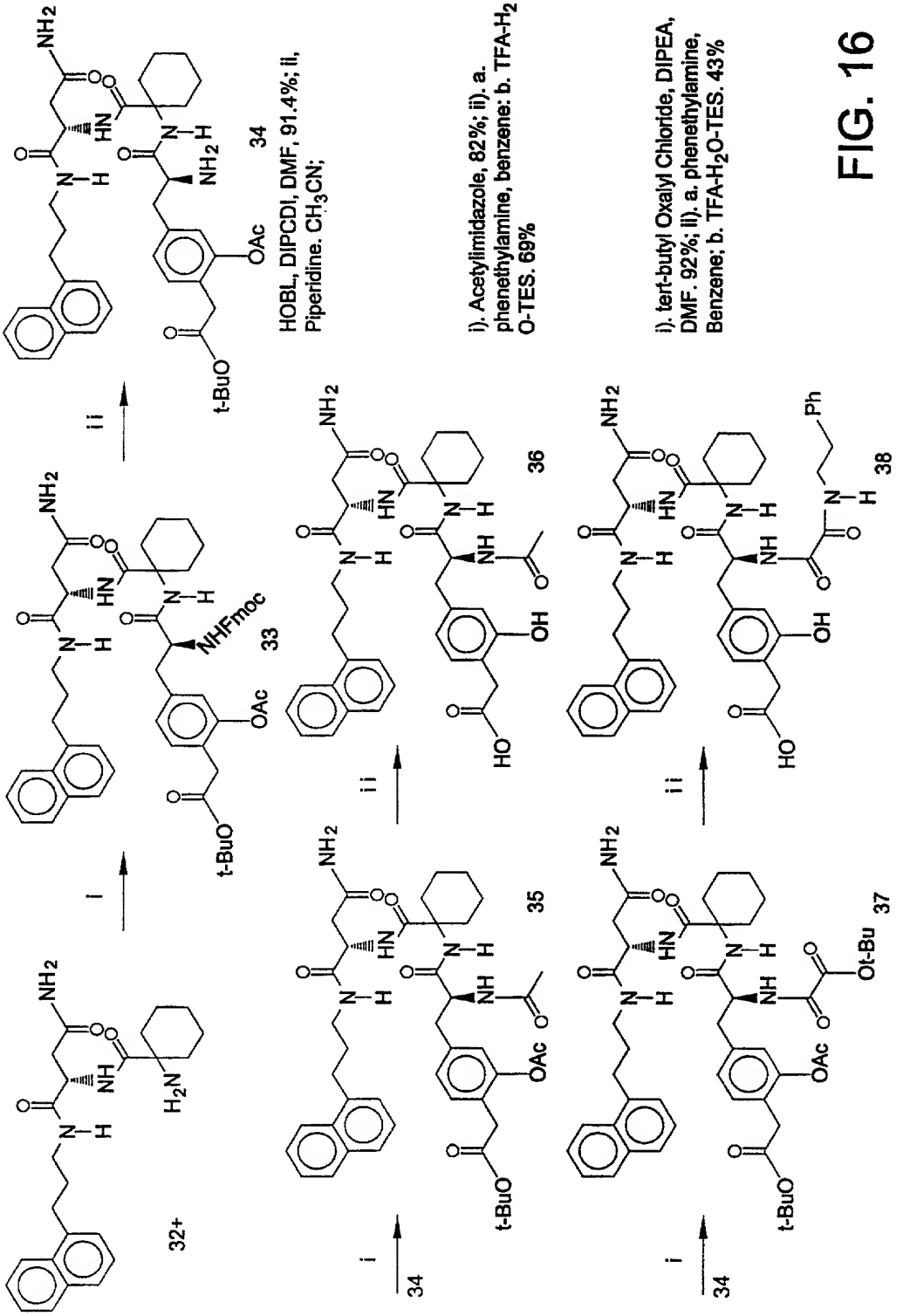

32+
i
33
ii
34
HOBL, DIPCDI, DMF, 91.4%; ii, Piperidine. CH_3CN;
i). Acetylimidazole, 82%; ii). a. phenethylamine, benzene: b. TFA-H_2 O-TES. 69%
i). tert-butyl Oxalyl Chloride, DIPEA, DMF. 92%; ii). a. phenethylamine, Benzene; b. TFA-H_2O-TES. 43%
35
36
37
38

FIG. 16

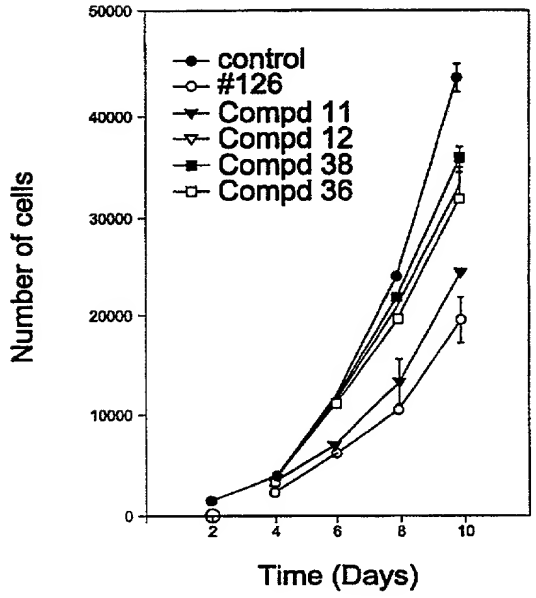


FIG. 17a

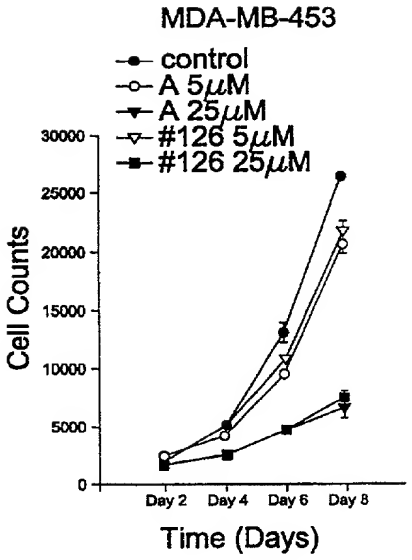


FIG. 17b

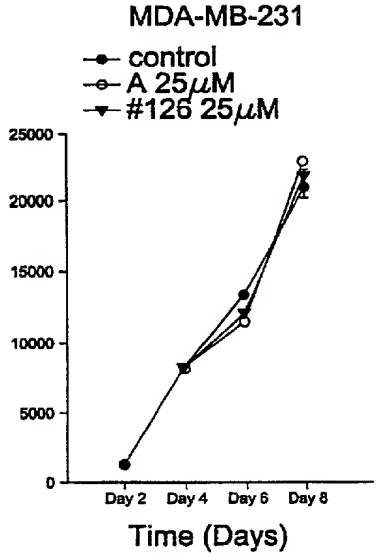
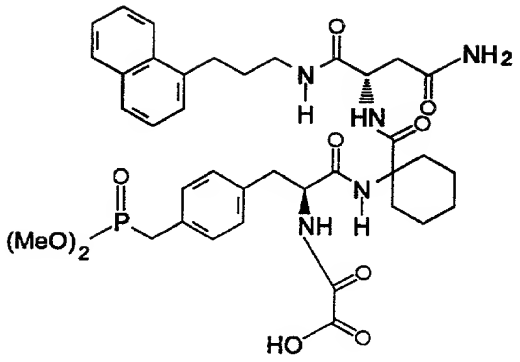
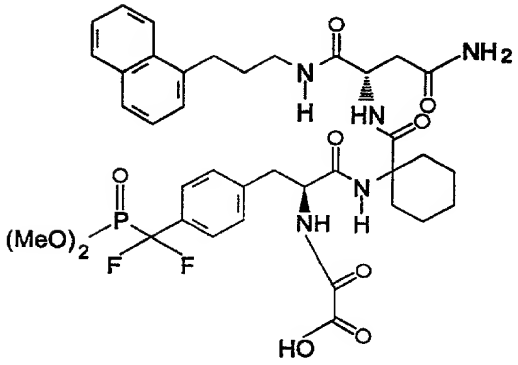


FIG. 17c

FIG. 18



Compd 126



Compd A

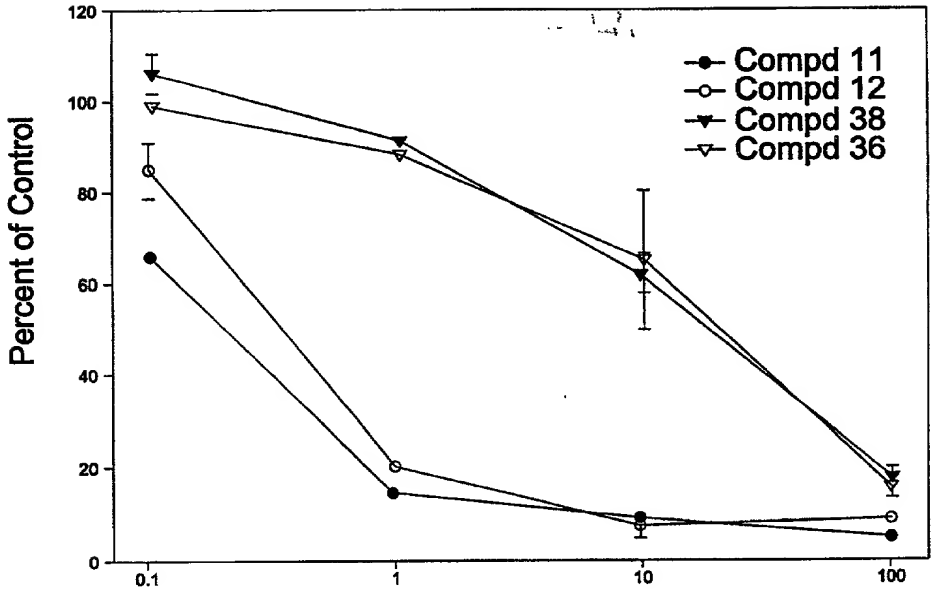

Compd 11
Compd 12
Compd 38
Compd 36
Percent of Control
120
100
80
60
40
20
0
0.1
1
10
100
Concentration of Grb2 Inhibitor (μM)

FIG. 19

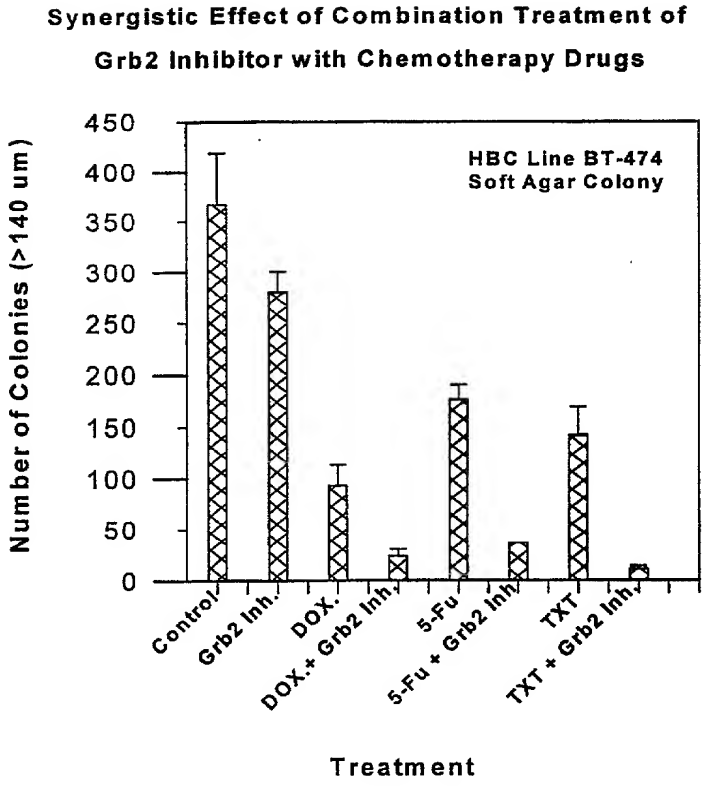

Synergistic Effect of Combination Treatment of
Grb2 Inhibitor with Chemotherapy Drugs
HBC Line BT-474
Soft Agar Colony
Number of Colonies (>140 um)
450
400
350
300
250
200
150
100
50
0
Control
Grb2 Inh.
DOX.
DOX.+ Grb2 Inh.
5-Fu
5-Fu + Grb2 Inh.
TXT
TXT + Grb2 Inh.
Treatment

FIG. 20

PATENT
Attorney Docket No. 401371

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

This declaration is of the following type:

☐ original ☐ design ☐ supplemental
☒ national stage of PCT
☐ divisional ☐ continuation ☐ continuation-in-part

My residence, post office address, and citizenship are as stated below next to my name. I believe I am the original, first, and sole inventor *(if only one name is listed below)* or an original, first, and joint inventor *(if plural names are listed below)* of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PHENYLALANINE DERIVATIVES

the specification of which:

☐ is attached hereto.
☐ was filed on as Application No. and was amended on *(if applicable).*
☐ was filed by Express Mail No. *as Application No. not known yet*, and was amended on *(if applicable).*
☒ was filed on March 23, 2000 as PCT International Application No. PCT/US00/08231 and was amended on *(if any).*

I state that I have reviewed and understand the contents of the specification identified above, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to the patentability of the application identified above in accordance with 37 CFR 1.56.

I claim foreign priority benefits under 35 USC 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent, utility model, design registration, or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter and having a filing date before that of the application(s) from which the benefit of priority is claimed.

PRIOR FOREIGN PATENT, UTILITY MODEL, AND DESIGN REGISTRATION APPLICATIONS						
COUNTRY	PRIOR FOREIGN APPLICATION NO.	DATE OF FILING (day,month,year)	PRIORITY CLAIMED			
				YES		NO
				YES		NO
				YES		NO

In re Appln. of BURKE, Jr ET AL.
Attorney Docket No. 401371

I claim the benefit pursuant to 35 USC 119(e) of the following United States provisional patent application(s):

PRIOR U.S. PROVISIONAL PATENT APPLICATIONS, BENEFIT CLAIMED UNDER 35 USC 119(e)	
APPLICATION NO.	DATE OF FILING (day,month,year)
60/126,047	March 23, 1999

I claim the benefit pursuant to 35 USC 120 of any United States patent application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this patent application is not disclosed in the prior patent application(s) in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose material information as defined in 37 CFR 1.56 effective between the filing date of the prior patent application(s) and the national or PCT international filing date of this patent application.

PRIOR U.S. PATENT APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S., BENEFIT CLAIMED UNDER 35 USC 120					
U.S. PATENT APPLICATIONS			Status *(check one)*		
U.S. APPLICATION NO.	U.S. FILING DATE		PATENTED	PENDING	ABANDONED
1.					
2.					
3.					
PCT APPLICATIONS DESIGNATING THE U.S.			Status *(check one)*		
PCT APPLICATION NO.	PCT FILING DATE (day,month,year)	U.S. APPLICATION NOS. ASSIGNED (if any)	PATENTED	PENDING	ABANDONED
4.					
5.					
6.					

DETAILS OF FOREIGN APPLICATIONS FROM WHICH PRIORITY CLAIMED UNDER 35 USC 119 FOR ABOVE LISTED U.S./PCT APPLICATIONS				
ABOVE APPLICATION. NO.	COUNTRY	APPLICATION NO.	DATE OF FILING (day,month,year)	DATE OF ISSUE (day,month,year)
1.				
2.				
3.				
4.				
5.				
6.				

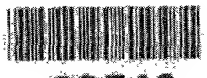
In re Appln. of BURKE, Jr ET AL.
Attorney Docket No. 401371

As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

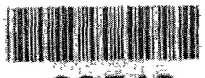
Robert Benson, Reg. No. 33,612
Steven M. Ferguson, Reg. No. 38,448
Stephen L. Finley, Reg. No. 36,357
James C. Haight, Reg. No. 25,588
John Peter Kim, Reg. No. 38,514
Richard U. Rodriguez, Reg. No. 45,980
Susan S. Rucker, Reg. No. 35,762
David R. Sadowski, Reg. No. 32,808
Marlene Shinn, Reg. No. 46,005
Jack Spiegel, Reg. No. 34,447

all of National Institutes of Health, Office of Technology Transfer, Box OTT, Bethesda, Maryland 20892-9902, Telephone (301) 496-7056.

Please recognize Leydig, Voit & Mayer, Ltd. as Associate Attorneys in this case: Customer No. 23548.



I further direct that correspondence concerning this application be directed to Leydig, Voit & Mayer, Ltd.: Customer Number 23548.



I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of **sole or first inventor:** Terrence R. BURKE, JR.

Inventor's signature Terrence R Burke Jr.

Date 9/18/2001

Country of Citizenship: US

Residence: Bethesda, MD
(city/state or country)

Post Office Address: 7400 Lakeview Drive, #410, Bethesda, MARYLAND 20817
(complete mailing address)

Full name of **second joint inventor,** if any: Yang GAO

Inventor's signature _____

Date _____

Country of Citizenship: CHINA

Residence: Branford, CT
(city/state or country)

Post Office Address: 62 Montoya Circle, Branford, CT 06405
(complete mailing address)

In re Appln. of BURKE, Jr ET AL.
Attorney Docket No. 401371

As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Robert Benson, Reg. No. 33,612
Steven M. Ferguson, Reg. No. 38,448
Stephen L. Finley, Reg. No. 36,357
James C. Haight, Reg. No. 25,588
John Peter Kim, Reg. No. 38,514
Richard U. Rodriguez, Reg. No. 45,980
Susan S. Rucker, Reg. No. 35,762
David R. Sadowski, Reg. No. 32,808
Marlene Shinn, Reg. No. 46,005
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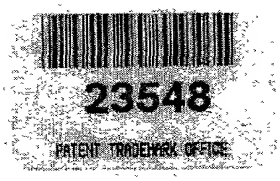
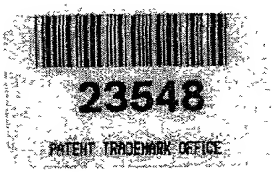
all of National Institutes of Health, Office of Technology Transfer, Box OTT, Bethesda, Maryland 20892-9902, Telephone (301) 496-7056.

Please recognize Leydig, Voit & Mayer, Ltd. as Associate Attorneys in this case: Customer No. 23548.



I further direct that correspondence concerning this application be directed to Leydig, Voit & Mayer, Ltd.: Customer Number 23548.



I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of **sole or first inventor:** Terrence R. BURKE, JR.

Inventor's signature _____

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Full name of **second joint inventor,** if any: Yang GAO

Inventor's signature Yang Gao

Date 09/14/2001 Country of Citizenship: CHINA

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(city/state or country)

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Full name of **third joint inventor,** if any: Zhu-jun YAO

Inventor's signature _____

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Full name of **fourth joint inventor,** if any: Dajun YANG

Inventor's signature _____

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NIH - Declaration (Rev. 6/12/2001)

In re Appln. of BURKE, Jr ET AL.
Attorney Docket No. 401371

Full name of **third joint inventor,** if any: Zhu-jun YAO

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Date 9/24/2001

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NIH - Declaration (Rev. 6/12/2001)